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GONAD GRAFTS IN EMBRYONIC CHICKS AND THEIR RELATION TO SEXUAL DIFFERENTIATION.*

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WITH FOUR PLATES.

CONTENTS

1. Introduction.

THE present series of experiments were designed with a view to substantiate, if possible, the claim made by Minoura in 1921, that the effect of grafting ovary or testis on to the chorio-allantoic membrane of the developing chick embryo is the production, in the host, of modifications in the character of the developing gonads and the associated ducts towards an intersexual type. Such modified chicks were referred to as intersexes and their causation explained by postulating, that in effect, the graft

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elaborates a specific secretion or hormone. This substance obtaining access to the tissues of the host through the common circulation acts, in the case of a testis graft on female chick, on the developing urogenital organs and produces a modification in the development of these organs approximating them to the male type. A similar explanation served in the case of male embryos, whose gonads and accessory sexual ducts were modified in the female direction through the activity of a specific secretion from the ovarian grafts.

As is well known in cattle the female in twins of the opposite sex is frequently sterile. Willier, who has made a complete histological study of the gonads from these sterile females, has shown that the gonad is primarily an ovary which has been modified to a more or less greater extent in the direction of a testis. The sexual ducts are male-like and the external genitalia, though usually female in type, may be modified also in the direction of the male.

The theory put forward by Lillie to explain these cases is briefly, that the sex hormones elaborated by the gonad of the male co-twin obtain access to the tissues of the female embryo through the anastomosing blood vessels of the fœtal membranes and produce a modification in the development of the sexual apparatus of the female in the male direction. Such a theory of course does not readily lend itself to experimental verification.

For the study of growth and differentiation of grafted tissues many workers have shown that the embryonic membranes of the developing chick offer exceptional facilities owing to the rapid vascularisation and incorporation of the graft.

This was the experimental method adopted by Minoura in order to reproduce artificially in the chick those abnormalities which occur naturally in the case of the female co-twin in cattle. A piece of testis or ovary was grafted on to the chorio-allantoic membrane of the developing chick. In favourable cases rapid vascular connection between the graft and the host was established. Any specific secretion of the implanted gonad was, therefore, through the common blood supply, accessible to the tissues of the host.

In the course of the research, however, one difficulty presented

itself. To quote from Minoura, "The interpretation of the intersexual individuals is rendered very difficult, since we do not know with certainty the sex of the embryos on which the graft was implanted." To obviate the difficulty in the present series of experiments, birds with a single sex-linked character difference were crossed and the eggs obtained were used as experimental material. This renders the determination of the sex of the embryo by an external examination an easy matter.

The facts that, primarily, the work of Minoura provides the only experimental evidence substantiating the theory of Lillie on the free-martin in cattle and therefore a confirmation of his results is required; and secondly, that in the present series improvements in technique have been devised, are held to offer sufficient justification for a repetition of this work.

2. Material.

As previously noted, the eggs used to provide the experimental material for this research were obtained from a sex-linked cross. The particular cross used was Light Sussex females × Rhode Island Red males, and the sex-linked character involved was the colour of the plumage. The down colour of the male chick is white and that of the female reddish-yellow. Sex can be determined by the down colour of the embryo as early as the tenth day of incubation. The chicks used to provide the tissues for grafting were, for the most part, closely related to the experimental ones and had been hatched in the laboratory. Some of the older birds used as a source of material for grafting were, however, unrelated to the embryos on which such tissues were grafted.

3. Method.

After incubating for seven days the egg for operation was removed from the incubator and candled. At this stage in the development of the embryo the position of the main blood vessels was clearly discernible. A mark was made on the shell over the intersection of two large vessels and the surface of the shell in this region well sterilised by rubbing with a piece of cotton-wool soaked in 70 per cent. alcohol. The removal of a piece of the shell was found to be done most

expeditiously and without injury to the underlying shell membrane by the use of a small circular saw, 1 cm. in diameter, attached to a dental drill worked by a small electric motor. The fragments of the shell were brushed off and the egg placed on a pad of cotton-wool over the candling box to minimise the cooling during the progress of the operation. A flap of the shell membrane was carefully lifted up and the piece of tissue to be grafted was placed on the chorio-allantoic membrane in the intersection between two of the larger blood vessels. It should be noted that no intentional injury to the embryonic membranes was attempted when grafting, but the tissue was placed on to the membrane as carefully as possible. The shell membrane was replaced and the opening in the shell sealed with a piece of sterilised paper previously immersed in fresh egg albumen. The egg was then returned to the incubator and not rotated for a period of eighteen hours. At the end of this time the egg was turned as usual twice daily.

The chick used to supply the grafting material was killed by decapitation, skinned, and placed in a sterilised petrie dish when the abdominal cavity was opened up; the dish was then covered and set aside until the egg was ready to receive the tissue to be grafted. The usual precaution of sterilising all instruments and dishes used during the operation was taken.

Out of each batch of eggs many were incubated normally and served as controls. In others the square piece of shell was removed and the shell membrane lifted up as if in preparation for the implantation of a graft. The membrane was then replaced in position and the opening sealed as described previously.

4. Age of Embryos at Grafting.

The seventh day from the beginning of incubation of the egg was chosen as the most suitable age to implant tissue on to the chorio-allantoic membrane of the developing embryo. At this stage the ectoderm of this membrane is supplied with a well-developed vascular network, and large blood vessels permeate the mesoderm ensuring rapid vascularisation and incorporation of the graft. The developing gonads at this stage have reached the end of the period of sexual indifference and sexual

differentiation has begun. From this time onwards the epithelial cords of the first proliferation begin to degenerate in the female—in the male they are destined to give rise to the spermatic tubules. The conditions for the production of successful grafts at earlier stages in the development of the chick are unfavourable, owing to the fact that the blood vessels of the fœtal membranes are not sufficiently well developed to ensure rapid vascularisation of the piece of grafted tissue. In this series of experiments no embryos were used as hosts for grafted tissue after the ninth day of incubation.

5. Age of Grafted Tissue.

The age of the grafted gonad tissue varied from that of 14th-day embryos up to tissues from cockerels or pullets ten weeks old. The size of the piece of grafted tissue was usually one quarter to a whole embryonic gonad, and in the case of the older tissue about 2 mm. square.

Altogether 540 embryos were used as hosts for the reception of grafted tissue. In the majority of cases the graft consisted of ovary or testis. Pieces of thyroid, liver, spleen, heart, lens, Wolffian body, adrenal, kidney, pancreas, and gall bladder were also used as grafting material; these latter were only used to ascertain whether the above-mentioned tissues had any effect on the development of the gonads or the urogenital ducts of the chick embryo on which they were grafted. The effect on the chick embryo as a whole, or on any part not connected with the sexual apparatus, has not been taken into consideration in the scope of this paper.

6. Examination of Operated Eggs.

Most of the eggs were removed from the incubator for examination at the seventeenth day of incubation; a few were opened on the fourteenth, fifteenth, and sixteenth day. The longest period that the graft remained on the chorio-allantoic membrane was ten days, and the shortest period seven days.

At the end of the necessary period of incubation the eggs were removed from the incubator and the paper sealing the opening in the shell scraped off. The shell was then carefully chipped away from the opening until half the egg was

uncovered. It was found that many of the eggs had become infected and had failed to develop. In the living embryos removal of the shell membrane exposed to view the position of the graft. In all cases a large white or pinkish growth, which varied greatly in size in different embryos—in some it was nearly 1 cm. in diameter—was seen, situated on the membrane at the point of implantation of the grafted tissue. The presence of this growth was demonstrated not only in the case where grafts were made but also in those embryos in which the operative technique was carried out up to the point of lifting the shell membrane, but where the egg was re-sealed without an actual graft being made. The grafted tissue may sometimes be seen situated in the centre of the mass, but it was found on histological examination of sections of the growths that in many cases grafted tissue could not be located. It is therefore evident that the presence of the growth does not indicate the presence of grafted tissue, and also that even when the grafted tissue can be located in the growth, the relative size of the growth is not an indication of the rate of growth in the various grafts, but really measures the amount of damage done to the chorio-allantoic membrane at the time of grafting.

Histologically the growth (fig. 6) consisted of a mass of inflammatory tissue, myxomatous mesenchyme with intricate network of capillaries, the walls of which showed layers of proliferating endothelial cells. The darker rounded areas (fig. 6) were infoldings of the surface epithelium which have been cut across tangentially. The dark central zone from which the capillaries are seen to radiate outwards is probably the zone of origin of the new-formed blood vessels. The spaces between the meshes formed by the anastomosing protoplasmic processes of the mesenchyme cells were normally filled with inflammatory exudate and a few polymorph cells.

The histology of the growth confirms the view that the pinkish mass of tissue results from an injury to the fœtal membrane at the time of grafting. The growth was dissected off the membrane and removed to a tube of fixative. (The growths and gonads obtained during the course of the experiments were fixed in Allen's modification of Bouin, and after

sectioning the tissues were stained by Heidenhain's iron-alum hæmatoxylin method). The egg content was poured out into a dish and a careful search of the chorio-allantoic membrane made. It was noted in several instances that where the presence of grafted material could not be clearly demonstrated in the centre of the growth, careful search of the chorio-allantoic membrane revealed the graft attached to the membrane at a spot some distance from the pinkish mass of the growth. Many of these grafts were quite satisfactory. Vascular connections to the graft were noticed and microscopical examination proved the presence of healthy grafted tissue.

The embryo was then dissected, the urogenital system examined macroscopically, and the gonads fixed for histological study.

Before proceeding to the description of the experimental embryos obtained and the results of the histological study of the growths and gonads, the actual experimental data will be given.

TABLE I.

Total number of eggs operated on	540*	Percentage of embryos living	34·1
Total number of living embryos obtained	184	Sex-ratio of living embryos	93 ♂ : 91 ♀

TABLE II.

Total number of testis grafts	233	Total number of ovary grafts	168
Embryos living after testis implantation	91	Embryos living after ovary implantation	59
Percentage living = 38·9		Percentage living = 34·8	
Male embryos living after—		Female embryos living after—	
Testis grafts	44	Testis grafts	47
Ovary grafts	27	Ovary grafts	32
Total	71	Total	79

* The total number of eggs operated on included, as well as ovarian and testicular grafts, all those tissues mentioned previously, namely, thyroid, adrenal, pancreas, spleen, gall-bladder liver, Wolffian body, kidney, lung, lens, and heart tissue. The eggs used as controls, whether incubated normally or subjected to the operative technique up to the point of lifting the shell membrane and then sealing without the implantation of grafted tissue, do not figure in the tables.

The percentage of living embryos obtained after operation is not abnormally low if we take into account the severity of the technique and the exposure of the egg to the possibility of infection during the operation.

7. Sex-ratio of the Embryos obtained after Operation.

In the fowl the sex-ratio normally reveals a slight excess of females. The number of males per thousand females as ascertained by various authors is as follows: 806 (Field), 947 (Darwin), 944 (Pearl). In these experiments the sex-ratio in the chicks surviving after operation, as shown by the tables, may be taken as normal. Thus the power of a chick to survive the operation is independent of the sex of the individual. Where gonad tissue was grafted, either testis or ovary, no significant difference in rate of mortality was shown in the two sexes.

8. The Development of the Urogenital System in the Normal Embryo.

Gonad.—The gonads are formed early in embryonic life from a thickening of the cœlomic epithelium in the angle between the mesentery and the mesonephros. They soon assume the form of ridges lying longitudinally along the inner face of the Wolffian bodies. The formation of the genital ridge results from the proliferation of the sub-epithelial mesenchyme. The germinal epithelium is composed of tall cylindrical cells between which are found oval or rounded cells—the primordial germ cells. After this point the germinal epithelium gives rise to local thickenings. These grow rapidly, push into the substroma, and gradually lose connection with the germinal epithelium giving rise to a network of sub-epithelial cellular cords. This stage marks the end of the period of sexual indifference, for up to the seventh day (according to Firket) it is not possible to determine whether the embryonic gonad will develop into ovary or testis. The epithelial cords abort in the female and are replaced by another proliferation at the eleventh day of epithelial cords from which the oöcytes of the functional ovary are derived. In the male, however, there is only one proliferation of sexual cords from the germinal epithelium. These enlarge and anastomose to form a sub-epithelial network of tubules from which ultimately the mature spermatic tubules are derived. In the right gonad of the female the medullary cords abort and are not replaced by a second proliferation of epithelial

cords as in the case of the left gonad. The microscopical changes taking place in the early period in the development of the gonad become apparent macroscopically at approximately the eighth day of incubation.

Plate I. gives critical stages in the development of the uro-genital organs of the embryonic chick. In fig. 1 a stage of sexual indifference is shown. The gonads are thin elongated organs situated anteriorly on the median ventral surface of the Wolffian body. Wolffian and Müllerian ducts are present on both sides. Further development of the uro-genital system marks the end of the period of sexual indifference. From now on the gonads in the two sexes show considerable variation in size and shape. In the female the left ovary increases in size and becomes somewhat flattened. The right ovary regresses as development proceeds, until at the time of hatching a mere trace of the gonad is usually to be seen, lying obliquely across the anterio-ventral surface of the disappearing Wolffian body. The cases illustrated are from typical embryos; there is, however, comparatively wide variation in the rate of regression of the right gonad. As previously described, no secondary proliferation of sexual cords takes place in the right ovary of the female, and the abortion of the primary sexual cords accounts for the regression of this organ. Some growth in size of the right gonad takes place during the second week of incubation.

In the male, the gonads develop more or less equally, though the right testis is frequently somewhat smaller than the left. The testes do not become flattened as in the case of the ovary. It is worthy of note that, as development proceeds, the anterior end of the right testis tends to bend to the right and assume an oblique position with regard to the Wolffian body in the same way as the right ovary. The change in position of the degenerating right ovary and of the anterior half of the right testis may be due to a more rapid regression of the right Wolffian body in both sexes, caused possibly by differential blood supply to the urogenital organs on both sides of the body.

Müllerian Ducts.—The Müllerian ducts in the male embryo begin to degenerate usually about the twelfth day of incubation

(fig. 3), and by the fourteenth day completely disappear. In the female, the left Müllerian duct increases in size and at the twelfth day signs of a posterior swelling are visible. A slight increase in diameter of the right Müllerian duct at its posterior end is also noticeable. Soon after, degeneration of the right Müllerian duct from its anterior end takes place. The duct, however, does not completely disappear, the posterior portion still being apparent at the time of hatching. Finlay gives in his paper a figure showing that in exceptional cases this fragment retains the power of growth. The left Müllerian duct continues to develop and becomes a functional oviduct.

From the development of the urogenital system it is seen that the sex of an embryo can be determined macroscopically by examination of the gonads from at least the eighth day of incubation by: (1) the difference in shape between the gonads in the two sexes; (2) variation in size between the right and left ovary in the female.

As development proceeds, further sexual characteristics become apparent, owing to the complete disappearance of the Müllerian ducts in the male and the partial disappearance of the right Müllerian duct in the female.

Wolffian Ducts.—The presence of Wolffian ducts occurs in both sexes throughout embryonic life. In the female, they are quite prominent at the time of hatching, though possibly not quite as much as in the male. The difference in diameter in the ducts in the two sexes is too small, however, to be used as a distinguishing sexual characteristic in these experiments.

9. Urogenital System of Experimental Embryos.

The great majority of the embryos were examined at the seventeenth day of incubation, when the sexual characteristics of the two sexes were very well marked. As previously stated, the sex of any of the experimentally obtained embryos was identified without any possible doubt by an external examination of the plumage of the embryo. In the female the plumage colour of the chick was reddish-yellow, and in the male white. It was found on internal examination of the urogenital organs and accessory ducts of the experimental embryos, no matter whether gonads or any other tissue had been implanted, that

they showed no deviation from the normal structure peculiar to the sex as identified by an examination of the plumage, with two exceptions to be described.

Abnormal Embryos.—Of the two abnormal forms obtained, one was a control embryo and the other had received a gall-bladder graft. Externally the chicks were structurally abnormal. They were cross-beaked and possessed no osseous formation at the top of the skull. Judging by the plumage colour they are females and the internal examination confirmed this. The gonads in both the chicks were normal, but a peculiarity of the Müllerian ducts was exhibited in that the right Müllerian duct had attained as high a stage of development as the left. Those who use incubators extensively know that such abnormalities are somewhat frequently met with, and result from a rise of temperature a few degrees above the optimum due to some error in the regulating mechanism.

As a general rule the appearance of the organs of the experimental chicks is unhealthy. The liver is green instead of reddish-brown, and the green-bile pigment is found to colour even the Wolffian body. Apart from the two abnormal forms described above no abnormalities of the urogenital system were produced in the presence of grafted tissue, whether the material used for grafting had been testis, ovary, or any other of the tissues mentioned.

Histology of the Growths.—It was found that on opening the egg at the seventeenth day of incubation for examination a pinkish mass of tissue at the opening of the shell was disclosed. In many cases this contained no evidence of grafted tissue and its histological features have been previously described. In some cases the presence of a graft was detected, attached to the embryonic membrane and well vascularised, at some distance from the mass of granulating tissue. The occurrence of such grafts was to be expected from the fact that, when grafting, the material was just placed on the embryonic membranes, and no attempt was made to localise the graft by breaking the external layer of ectoderm and embedding it in the mesenchyme.

The grafts are found embedded in the mesenchyme underlying the ectoderm of the embryonic membrane. They show

a continuous range from the presence of well-developed and healthy grafted tissue, with little or no areas of degeneration, passing through a stage in which the healthy grafted tissue is restricted to its peripheral areas, the centre being occupied by a degenerating mass of tissue, to that shown in fig. 7 in which no healthy grafted tissue can be discerned.

10. Histology of Gonad Grafts.

Testis Grafts.—The graft was normally found situated in the mesenchyme below the ectoderm of the chorion. Surrounding the graft there was a sheath of connective tissue corresponding to the normal tunica albuginea of the testis. Just outside this was a vascular layer composed of numerous blood vessels and capillaries each of which was the centre of a large area of polymorpho-nuclear leucocytes. Occasionally, as in fig. 13, a large blood clot surrounded by a broad zone of large, foreign-body giant cells could be seen in close proximity to the graft. This evidently results from a rupture of one of the blood vessels of the embryonic membrane during the implantation of the graft. In good grafts the seminiferous tubules were quite normal and mitotic figures were numerous, denoting active growth. The interstitial tissue was apparently normal but polymorpho-nuclear cells were numerous. In one or two of the grafts the deposition of large amounts of deeply stained granular pigment had taken place between the spermatic tubules. The writer is not prepared to attach any great significance to the presence of this pigment, as in the course of examining chicks used to provide grafting material the presence of a testis, either completely pigmented or pigmented in one area only, was not unfrequently met with. Fig. 7 shows an almost completely degenerated testis graft. The outlines of the tubules were still faintly visible, but in nearly all the basement membrane had broken down and the tubules were filled with a mass of debris in which only a few degenerate nuclei were found.

Ovary Grafts.—Very few successful ovarian grafts were obtained. The majority were highly necrosed and showed no evidence of growing gonad tissue. The successful graft was usually attached to the fœtal membrane of the host embryo by

means of the medullary cord region of the graft. The typical ovarian structure was retained, consisting of an outer layer, the cortex, and inner layer of degenerate medullary cords. The oöcytes were normal (fig. 22) and little evidence of degeneration was seen.

It was found that the percentage of successful testis grafts obtained far exceeded that of successful ovary grafts, independently of the sex of the embryo in which the graft was made. It is difficult to account for this, and it is suggested that the structure of the ovary may not be favourable for rapid vascularisation of the graft.

In Table III. details are given of the successful grafts of gonad tissue obtained on chicks of the opposite sex to those from which the grafting material was taken. All the cases listed had successful grafts, in that these contained, at the most, minute centres of necrotic material scattered through the bulk of healthy active gonad tissue.

TABLE III.

No. of Chick.	Sex.	Age when grafted.	Nature and Amount of Graft.	Age of Grafted Tissue.	Duration of Graft.
28	♀	7 days	⅓ testis	7 days	9 days
60	♀	7 ,,	½ ,,	4 ,,	10 ,,
62	♀	7 ,,	1 ,,	4 ,,	10 ,,
78	♀	7 ,,	⅓ ,,	7 ,,	10 ,,
109	♀	7 ,,	⅓ left ovary	3 ,,	9 ,,
118	♀	7 ,,	1 testis	14 day embryo	9 ,,
238	♀	7 ,,	1 ,,	18 ,,	10 ,,
266	♀	7 ,,	1 ,,	1 day	10 ,,
272	♂	7 ,,	⅓ left ovary	3 days	10 ,,
347	♂	7 ,,	½ ,,	17 day embryo	7 ,,
349	♀	7 ,,	½ ,,	17 ,,	7 ,,
383	♀	7 ,,	¾ testis	3 days	8 ,,
394	♀	7 ,,	½ ,,	7 ,,	7 ,,
411	♀	7 ,,	1 ,,	15 day embryo	9 ,,
425	♀	7 ,,	½ ,,	17 ,,	10 ,,
432	♀	8 ,,	½ ,,	17 ,,	9 ,,
434	♀	8 ,,	½ ,,	17 ,,	9 ,,
449	♀	7 ,,	½ ,,	1 day	10 ,,
456	♀	7 ,,	¼ ,,	2 days	10 ,,
469	♀	7 ,,	⅓ ,,	3 ,,	10 ,,
477	♂	7 ,,	⅓ left ovary	7 ,,	10 ,,
483	♀	7 ,,	½ testis	7 ,,	10 ,,
497	♀	9 ,,	2 mm. testis	70 ,,	8 ,,
498	♀	9 ,,	2 ,,	70 ,,	8 ,,
501	♀	9 ,,	2 ,,	70 ,,	8 ,,

The following is a brief description of the normal histological structure of the testis at the seventeenth day of incuba-

tion. The gonad is enclosed in a tunica albuginea several cells thick, consisting of loose connective tissue elements with flattened elongated nuclei. Blood vessels occur at frequent intervals. The immature spermatic tubules are surrounded by a well-defined basement membrane lined with epithelial cells. Numerous primordial germ cells with large nuclei are present in the tubules and a great many of them are undergoing mitosis. No synaptic stages are to be seen. At this stage of development the tubules show signs of branching. The intertubular tissue is abundant and small blood vessels and hæmatopoietic foci occur occasionally throughout the mesenchyme.

No deviation from this normal type of structure was found in the testes of embryos obtained after ovarian grafts, but for an increase in the hæmatopoietic foci of the number of cells belonging to the myeloid group.

The normal ovary at the seventeenth day of incubation consists of an external layer of germinal epithelium; under this layer are situated the greatly enlarged cortical cords containing the definitive oöcytes, some of which are in various stages of synapsis, while others are in the resting stage. Owing to the enlargement of the cords the band of connective tissue between the germinal epithelium and the degenerating medullary cords has been almost completely obliterated. The medullary cords are all canaliculised, the process being more advanced at the proximal end, where they are represented by an open network in the meshes of which are found islets of luteal cells; these are also numerous at the distal end. Hæmatopoietic foci occur as in the testis.

There was no deviation from this type in the structure of ovaries, embryos obtained after testis grafts, except that, as in the testis, the cells belonging to the myeloid group were increased in number.

II. Discussion.

The results of the experiments given in this paper do not lend any support to the view that in the embryonic chick a grafted gonad of the opposite sex secretes definite hormones which, obtaining entrance to the body of the host, cause the differentiation of the urogenital system to be modified so that

intersexual forms result. The results do not agree with those recorded by Minoura. Critical examination of Minoura's method and his interpretation of results shows the latter to be open to serious criticism.

In order to demonstrate successfully the production of intersexual forms due to the action of specific sex-hormones secreted by a gonad graft, several essential conditions must be fulfilled :—

(1) If the theory of the modification of embryos by the action of definite sex-hormones elaborated by the grafted gonad is to be accepted, then the presence of healthy grafted tissue on the chorio-allantoic membrane must be adequately demonstrated. The fact that on opening the egg at some period subsequent to grafting a white or pinkish mass may be observed having vascular connection with the embryonic membrane, has been shown to bear no direct relation to the graft ; it has also been shown that even if the graft is present the size of the growth does not indicate activity in the growth of the graft, but is merely the measure of the damage done to the delicate chorio-allantoic membrane during the operation. The fact that such growths are present when the operative technique is carried out up to the point of lifting up the shell membrane and sealing the opening in the shell without implanting grafting material, supports the view that the presence of such tissue masses is a response of the injured membrane.

Minoura does not state whether all growths were histologically examined, and in his description of the intersexual forms obtained no histological evidence of the presence of grafted tissue, healthy or necrotic, was given except in two cases, one a testis and the other an ovary graft.

(2) The original sex of the embryo on which the graft was implanted must be identified, as otherwise the interpretation of abnormal forms is necessarily rendered very difficult. The use as experimental material of eggs from a mating, the male and female offspring of which show a single sex-linked character difference, makes it possible to determine whether the experimental embryo is a genetic male or female. Such material was obtained for the experiments described in this paper, *i.e.* eggs

from Rhode Island Red males × Light Sussex females. The identification of the genetic sex of the embryo by an external examination was thus rendered infallible, even as early as the tenth day of incubation.

In the female there is in all embryos considerable variation in the degree of regression of the right gonad. Slightly atypical females with lesser regression than usual may not necessarily represent an intersexual condition. Similarly, the presence of Müllerian ducts associated with male type gonads may not indicate intersexuality: such individuals may be exceptional males in which growth and differentiation of Müllerian ducts has occurred; these ducts are present in male embryos during the early stages of development, but usually degenerate about the twelfth day of incubation. In the absence of evidence as to the genetic sex of the abnormal forms obtained by Minoura, it is possible that his cases may be classified under these two heads.

(3) It is essential to submit the gonads of abnormal embryos to careful histological examination. Minoura's brief histological description of the gonads of intersexual embryos does not indicate any striking deviation from the normal type: male type gonads, considered as ovary modified by testis graft, and female type gonads, regarded as testis modified by ovary graft, do not differ greatly from normal testis and ovary respectively. The presence of inactive or degenerating cells in the seminiferous tubules, and the presence of necrosis or partial disappearance of the cortical cords of the ovary could possibly be due to pathological conditions induced in the embryo by the presence of necrosed grafted tissue on the embryonic membrane. As previously described by the present writer, histological examination of embryos in which successful gonad grafts of the opposite sex were present did not reveal any deviation from the typical structure.

(4) The effect of the production of a number of intersexual forms on the sex ratio in the surviving chicks must be considered. It has been shown in the experiments described in this paper, that the normal sex ratio in the chicks living after the operation is maintained, practically equal numbers of males and females being obtained. If intersexual forms are produced

as a result of the action of testis grafts on female chicks, then a corresponding reduction in the number of unmodified females below the normal sex ratio may be expected. Similarly, in the case of ovary grafts, the production of intersexual forms resulting from the interaction between the grafts and the male embryos will reduce the number of unmodified males below the normal ratio.

Examination of Minoura's figures shows that out of 104 individuals living after testis grafts, 16 were classified as intersexual and therefore, presumably, were originally females whose sexual differentiation had been modified in the direction of the male by the action of the graft. Of the remaining chicks 52 were females and 36 were males, *i.e.* in spite of the production of 16 intersexual forms there was no decrease in the number of unmodified females below the normal. If it is considered, however, that most of the so-called intersexes were not females modified by the effect of the graft, but males in which an unusual development of the Müllerian ducts had occurred, then the original sex-ratio of the chicks would not show any appreciable divergence from the normal. In the chicks living after ovary grafts the relative numbers of the sexes are not so striking as in the case of chicks living after testis grafts. Out of the 83 chicks obtained 14 were classified as intersexual forms, the remainder consisted of 37 females and 32 males. The number of unmodified males obtained is therefore somewhat lower than would be expected in a normal population.

If we accept Minoura's interpretation of the original sex of his "intersexes," then in the case of the chicks with testis grafts the ratio of genetic males to genetic females was 36 : 68, while in the case of the chicks with ovary grafts the ratio of genetic males to genetic females was 46 : 37. These ratios are strikingly divergent, and statistical analysis shows that the odds against this divergence being due to random sampling are approximately 200 : 1. Indeed it is so unlikely that such divergence could arise by random sampling as to throw doubt on the validity of Minoura's identification of the original sex of his modified chicks.

Since the evidence produced by Minoura cannot be said

to demonstrate conclusively that the production of intersexual individuals was due to the action of the sex hormones of the graft, it seems desirable to consider the possible causes responsible for the occurrence of abnormalities in the reproductive system described in some of the embryos. Granting the occurrence of sexually abnormal forms, these can be divided into two classes.

(1) **Those not due to the Presence of the Graft.**—(*a*) *Environic.*—Fluctuations in temperature, moisture content, failure to turn the eggs daily during the process of incubation of the eggs, must all be considered as possible agents affecting adversely the normal development and differentiation of the embryo.

(*b*) *Genetic.*—Cases of naturally occurring intersexuality in fowls have frequently been described; in such individuals an ovary or ovo-testis is present on the left side and a testis on the right. This, according to Benoit, is due to insufficient functioning of the ovary at a very early stage in development, permitting the sexual cords of the first proliferation to develop into testicular elements before their regression has proceeded too far. This only takes place in the female, no evidence of intersexuality in the male having been recorded. This is not a likely cause of the occurrence of intersexual forms in such experiments.

(2) **Those due to the Presence of the Graft.**—It is impossible to determine whether the intersexual condition as described by Minoura was caused by the specific activity of the small active portion of the graft, or was due to the presence of the large necrotic growth on the embryonic membrane affecting normal differentiation of the urogenital system. In the experiments set forth in this paper it is shown that many healthy active grafts were obtained which did not in any way interfere with the normal processes of sexual differentiation. Operated eggs were examined in the majority of cases on the seventeenth day of incubation when the graft had been present on the fœtal membranes for ten days. Since at the seventeenth day of incubation the differentiation of the urogenital system is almost as complete as at the time of hatching, any abnormalities of the gonads or their associated

ducts are easily discernible. In all the cases listed in Table III. well-developed active grafts were present. If, as Minoura affirms, such grafts influence the sexual differentiation of the operated embryo, we should expect to find intersexual forms among the cases quoted. As stated above, however, such forms are entirely absent.

It may be argued that antagonism between the hormones produced by the graft and those produced by the host embryo (since at the time of grafting sexual differentiation from the indifferent stage has already begun) may have rendered ineffective the action of the graft hormone. This may possibly be the case, but it is not likely in view of the established fact that ovarian and testicular tissues can co-exist and exert their typical specific physiological influences in one and the same individual. Such artificial hermaphrodites have been induced only in a fully sexually differentiated individual and in such the conditions are not the same as in the embryo.

It has been claimed that the method of grafting gonads on to the chorio-allantoic membrane of the developing chick reproduces the conditions obtaining in the case of the free-martin in cattle (Lillie), where, owing to anastomosis of the blood vessels, a common circulation is established between bisexual embryos. The two cases, however, are not analogous. In the latter, it is the multiple secretions of all the endocrine organs of the male embryo which are poured into the common blood stream and circulated in the body of its female co-twin, while in the case of the egg grafts, the isolated organ exerts only its own specific physiological influence. Moreover, in the interpretation of the bovine free-martin it is assumed with reason that phenotypic maleness and femaleness depend upon the presence of functional testis and ovary respectively. Such interpretation does not explain why the male comes to possess testes, the female, ovaries. However, in the case of the fowl the sexes are genetically different, as is clearly shown by the facts of sex-linked inheritance. The cytological evidence is as yet insufficient to enable us to demonstrate the exact difference in the chromosome complex of the two sexes, but it is established that the female is the heterogametic sex. In the beginning of the differentiation of the embryonic gonad (either XX or XY

in constitution) there is an indifferent stage the subsequent differentiation of which into testis or ovary is determined by the type of physiological environment (established by specific inheritance) in which it happens to be situated. In other words, the gonad is moulded by its environment, and does not mould it except as regards the late developing secondary sex characters.

This is best illustrated by the case of the ovary in the fowl. Results from experimental grafting of ovaries in very young chicks (Finlay, 1925) demonstrate that the ovary possesses a certain instability; the engrafted gonad in its further development may show the presence of definite spermatic tissue. A parallel instance is shown in the cases of naturally occurring intersexes, many of which have been recorded in scientific literature. A change in the physiological environment may cause a concurrent change in the structure and function of the gonad, so that from a typical ovary secreting presumably ovarian hormone, it is modified into a testis secreting a testicular hormone. It is interesting to note, however, that there is no evidence whatsoever that would lead us to suspect a similar instability in the case of the testis in the fowl. There is no case on record in which intersexual gonads were found in the male. It would thus seem that the production of intersexual forms by ovarian grafts in male individuals is highly improbable.

It has to be stated that at a later stage in the development of the gonads they condition the production and maintenance of the secondary sexual characters.

It is shown in the present paper that the evidence derived from a study of gonad implants on the developing chick does not support the contention that in the fowl the processes of sexual differentiation are capable of modification solely through the action of the gonadic implant.

12. Summary.

1. An experimental study on the effects of gonad grafts in the embryo chick was undertaken in an attempt to reproduce the results obtained by Minoura and to define the bearing of this experiment on Lillie's theory of the free-martin in cattle.

2. Eggs from a sex-linked cross were used so as to be able to identify the original sex of the embryos.

3. In the majority of cases the egg received the graft at the seventh day of incubation and was examined at the seventeenth day, at which stage the progress of sexual differentiation is almost as complete as at the time of hatching.

4. In all 540 embryos were operated on. Of this number 233 received testis grafts and 168 ovary grafts. Other tissues grafted were thyroid, adrenal, pancreas, spleen, gall-bladder, liver, Wolffian body, kidney, lung, lens, and heart.

5. The age of the grafted tissue varied from the fourteenth day of incubation up to ten weeks old.

6. Of the gonad grafts, 150 survived the operation, 71 ♂ and 79 ♀. 47 ♀ chicks were obtained after testis grafts, and 27 ♂ chicks were obtained after ovary grafts. Healthy active testis grafts were found in 20 ♀ and 5 ovary grafts in ♂.

7. The sex ratio in the surviving chicks showed no deviation from the normal.

8. Macroscopical examination of the urogenital system showed no deviation from the typical structure consonant with the sex of the chick as determined by its plumage colour.

9. Microscopical examination of the gonads revealed no abnormalities in histological structure.

10. These results lend no confirmation to the view that the process of sexual differentiation in the chick can be profoundly modified by the specific physiological activity of a gonad graft of the opposite sex.

11. Minoura's interpretation of his results are criticised.

12. The grafting of gonad tissue in the embryo chick does not reproduce experimentally the conditions existing in the bovine free-martin in that in the former the embryo is exposed to the specific action of the grafted gonad alone, whereas in the latter the female co-twin is exposed to the action of all the internal secretions from the male.

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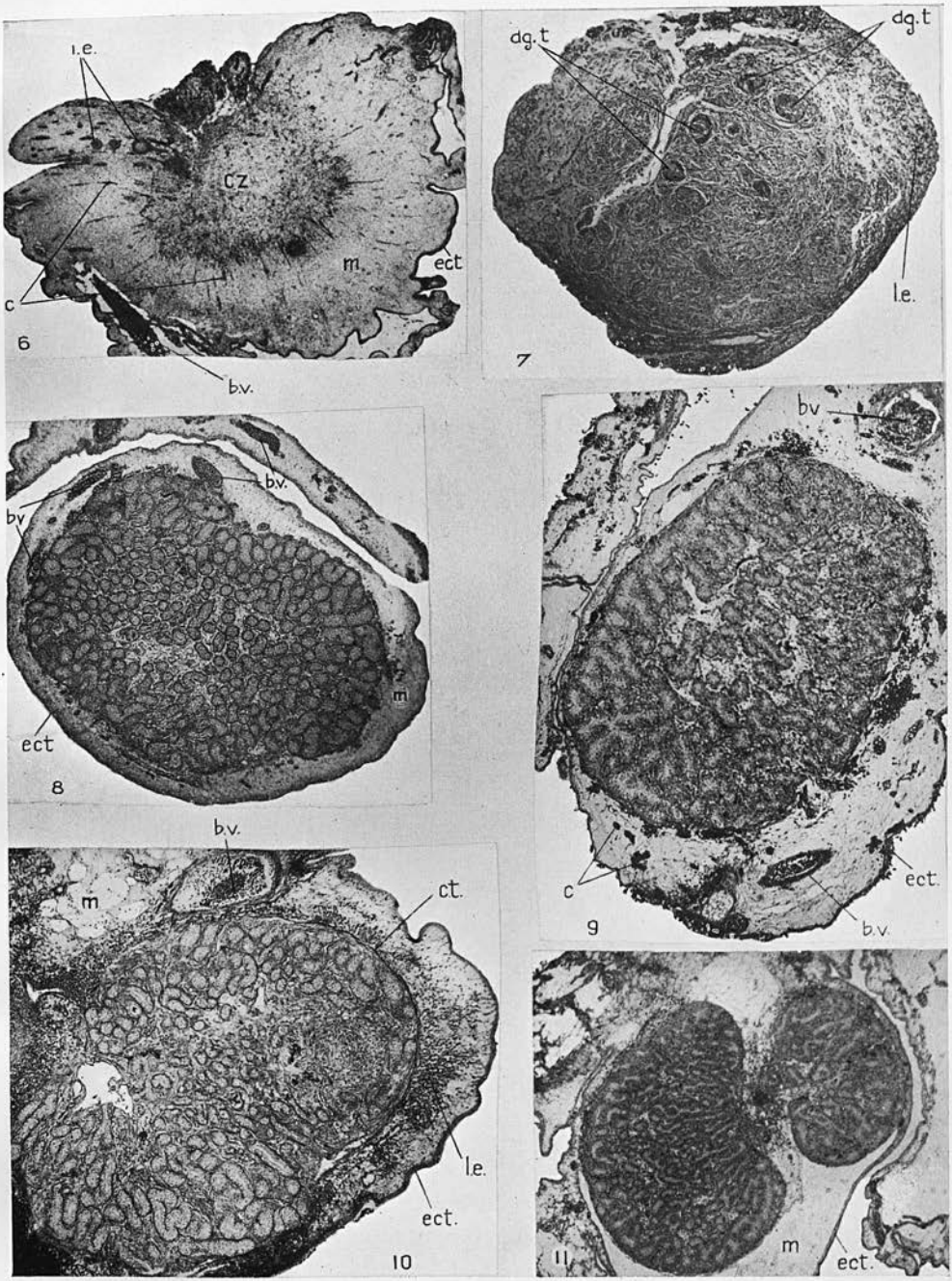
14. Description of Plates.

b.c. . . Blood clot.
b.v. . . Blood vessels.
c. . . Capillaries.
c.c. . . Cortical region.
c.t. . . Tunica albuginea of testis graft.
c.z. . . Zone of origin of new-formed blood vessels.
dg. . . Degenerate.
dg.t. . . Degenerate tubules in testis graft.
e. . . Erythrocytes.
ect. . . Ectoderm of chorion.
g. . . Gonad.
g.c. . . Zone of foreign body giant cells.
i.e. . . Invagination of surface epithelium.
l.e. . . Area of leucocytes.
m. . . Mesenchyme.
m.c. . . Medullary cord region.
m.d. . . Müllerian duct.
ooc. . . Oöcytes.
p. . . Pigment granules.
ur. . . Ureter.
w.b. . . Wolffian body.
w.d. . . Wolffian duct.

Female Line
Male Line
g.
w.b.
w.d.
m.d.
7th day
Indifferent stage
9th day
12th day
dg.m.d.
dg.m.d
ur
14th day.
ur?
dg.r.g.
w.d.
dg.m.d.
20th day
1
2
3
4
5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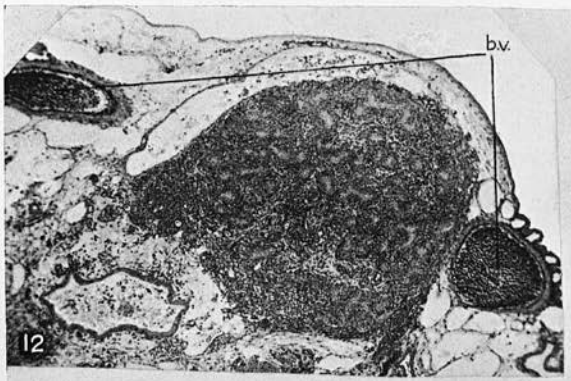
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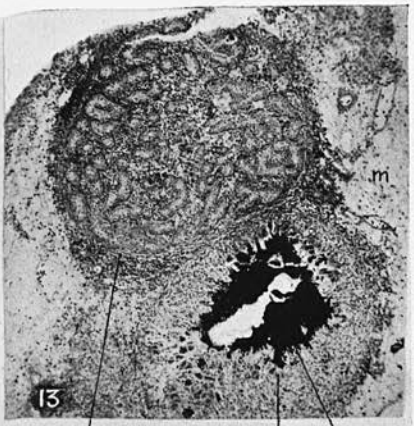
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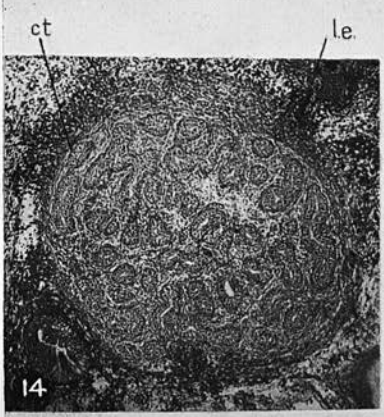
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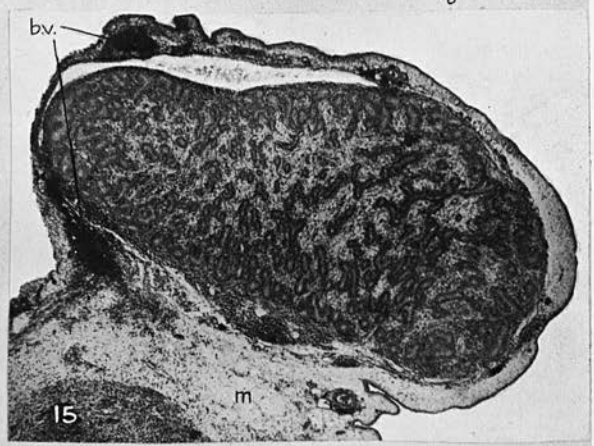
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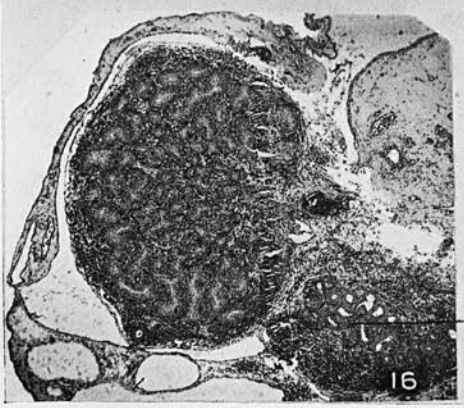
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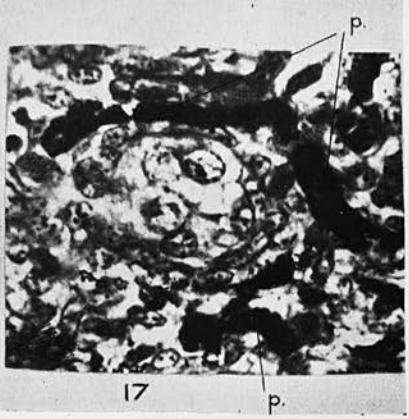
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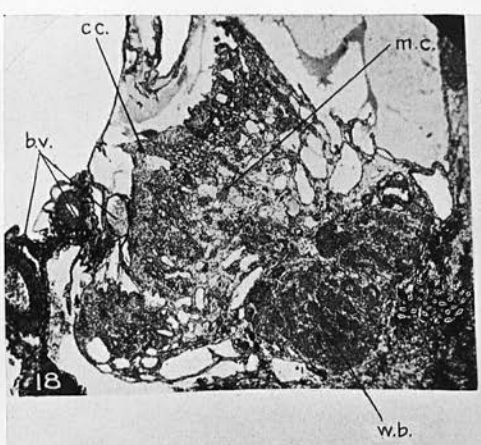
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b.v.
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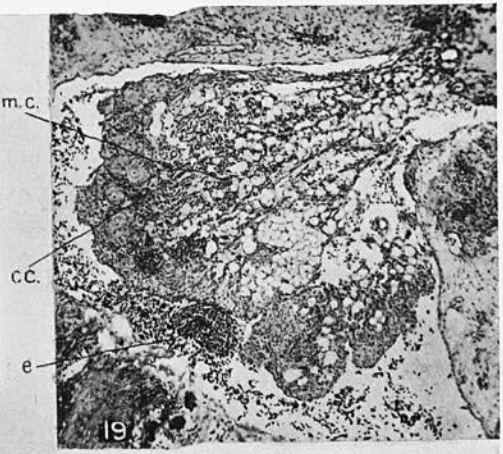
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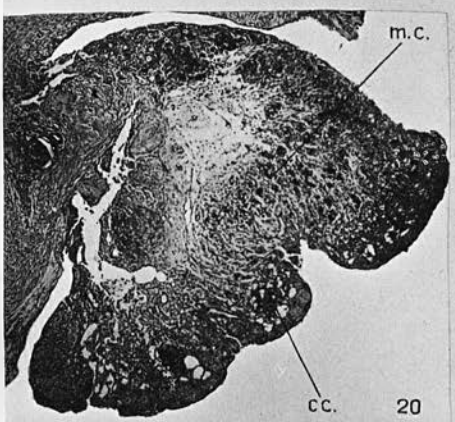
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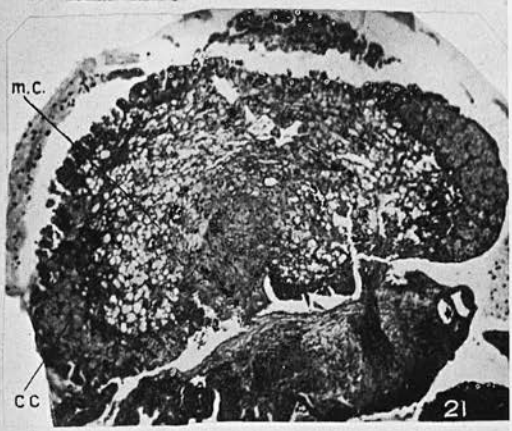
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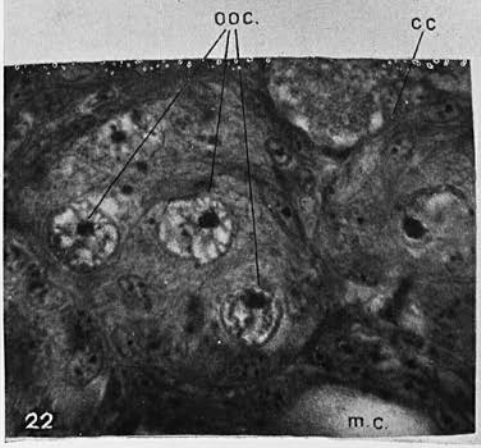
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m.c.

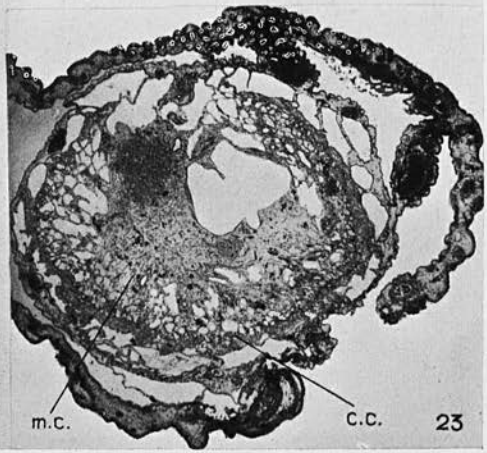
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PLATE I.—DEVELOPMENT OF THE UROGENITAL SYSTEM IN THE CHICK EMBRYO (actual size).

FIG. 1.—Embryo of 7 days. At this stage the sex of the embryo cannot be identified by macroscopic examination of the urogenital system.

FIG. 2.—Embryo of 9 days. (*a*) Female. Note flattened left ovary and smaller right ovary. (*b*) Male. Testes equal in size.

FIG. 3.—Embryo of 12 days. (*a*) Female. Bulbous swelling at cloacal end of left oviduct; right Müllerian duct shows very slight posterior swelling. Size difference between the two ovaries greatly increased. The right ovary is lying obliquely across the Wolffian body. (*b*) Male. Müllerian ducts show degeneration.

FIG. 4.—Embryo of 14 days. (*a*) Female. The right Müllerian duct degenerating. Ureters now distinct. Kidney developing. (*b*) Male. The ureters are distinct. Müllerian ducts absent. Note oblique position of anterior end of gonad (right) and development of kidney.

FIG. 5.—Embryo of 20 days. (*a*) Female. Remnant of right Müllerian duct and a trace of right ovary seen. The Wolffian bodies disappearing. Large flat left ovary. (*b*) Male. Two testes approximately equal in size.

PLATE II.—TESTES GRAFTS IN EMBRYONIC ♀ CHICKS.

FIG. 6.—Section of Growth (Chick 101) × 15.
FIG. 7.—Section of Testis Graft (Chick 84) × 55.
FIG. 8.— „ „ („ 498) × 40.
FIG. 9.— „ „ („ 434) × 50.
FIG. 10.— „ „ („ 501) × 55.
FIG. 11.— „ „ („ 411) × 35.

PLATE III.—TESTES GRAFTS IN EMBRYONIC ♀ CHICKS.

FIG. 12.—Section of Testis Graft (Chick 425) × 50.
FIG. 13.— „ „ („ 394) × 55.
FIG. 14.— „ „ („ 98) × 70.
FIG. 15.— „ „ („ 383) × 30.
FIG. 16.— „ „ („ 266) × 45.
FIG. 17.— „ „ („ 266) × 770.

PLATE IV.—OVARIAN GRAFTS IN EMBRYONIC ♂ CHICKS.

FIG. 18.—Section of Ovarian Graft (Chick 347) × 35.
FIG. 19.— „ „ („ 477) × 70.
FIG. 20.— „ „ („ 109) × 50.
FIG. 21.— „ „ („ 272) × 50.
FIG. 22.— „ „ („ 109) × 800.
FIG. 23.— „ „ („ 349) × 25.

Gonad Grafts in the Fowl

BY

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(From the Animal Breeding Research Department, The University, Edinburgh)

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University of Melbourne.

(From the Animal Breeding Research Department, The University, Edinburgh.)

With Four Plates.

CONTENTS

1. Introduction.

Although the fowl has been used extensively as experimental material for the allo-transplantation of reproductive tissue, as yet no detailed histological study of the grafted tissue has been made after its persistence for a considerable period in another body. The results described in this paper show that in such circumstances extensive changes in the structure of the grafted tissue occur. That such changes have not been reported by previous workers may possibly be due to the fact that in every case sexually mature gonadic tissue had been engrafted. The material used in the present investigation consisted of sexually immature reproductive tissue. The host and donor of the graft were in all cases of the same age, and the operation was performed soon after hatching (not later than the eleventh day).

I am greatly indebted to Dr G. F. Finlay for supplying me with the material upon which this study was made. I also wish to thank Dr F. A. E. Crew, Director of the Animal Breeding Research Department, and Dr H. B. Fell for much constructive criticism during the course of this study.

2. Material and Method.

The material was obtained from birds described by Finlay.* These were either pure-bred Brown Leghorn or chicks from a Silver Duckwing Leghorn ♀ × Brown Leghorn ♂ cross. The grafting was carried out between the second and the eleventh day, and the graft was in each case closely related to the host. The birds were examined post-mortem about a year after the transplantation and the material was immediately fixed in Bouin. Sections 7 μ in thickness were cut and stained by Heidenhain's iron-alum hæmatoxylin method. Altogether material from nineteen birds will be described.

In addition to the review of the histological features of the grafted tissue, two cases of an activated right gonad in the ovariotomised hen will be described and discussed.

Finlay formed the following experimental classes. The number of the individual is that given to it by Finlay.

Group 1.—Males with testis. Fowls Nos. 9 and 10.
Group 3.—Males with ovaries. Fowls Nos. 3, 15, 16, 17, 18.
Group 4.—Males with both testis and ovary. Fowls Nos. 4, 20, 21.
Group 5.—Females with testis. Fowls Nos. 5, 22, 23, 24.
Group 6. — Spayed females. Fowls Nos. 6, 26.
Group 8.—Females with both testis and ovary. Fowls Nos. 8, 29, 30.

(Finlay's Group 2 was composed of gonadectomised males and Group 7 of normal females.)

Since on post-mortem examination certain individuals of Groups (3) and (5) were found to have been incompletely gonadectomised, regeneration of the original gonad having taken place, the above classification is not strictly accurate.

3. Description of Cases.

Group 1. Males with Testis—(*a*) *Fowl No.* 9.—Castrated when eleven days old; three testes from half-brothers grafted subcutaneously on the left side, two on the right. Killed when one year old. Post-mortem examination revealed on the left side a subcutaneous testis nodule about one-quarter the size of a normal testis, also a smaller nodule about the size of a pea. No trace of gonadic tissue inside the body cavity.

* See "Studies on Sex Differentiation in the Fowl," by G. F. Finlay, in this number of this *Journal*.

Histology.—The two grafts presented the same histological appearance. They were surrounded by a thick fibrous tunica albuginea enclosing the mass of spermatic tubules. Two types of tubules of distinct histological appearance were present (fig. 1). (1) Large swollen tubules similar in appearance to normal seminiferous tubules undergoing active spermatogenesis. All meiotic stages were represented and numerous packets of fully developed spermatozoa were found in the central area of the tubules. (2) The tubules of the second type were not so large, and contained within the well-defined basement membrane a single layer of epithelial cells. The nuclei of these cells contained one or two deeply staining nucleoli and scanty chromatin network. The cytoplasm of the epithelial cells was fibrillar and fine strands stretched across the central lumen. Some of the tubules in the vicinity of those undergoing active spermatogenesis contained in the central lumen numerous fully developed spermatozoa. Tubules of this type occurred together in distinct areas and formed about two-thirds of the graft. The intertubular spaces between the active tubules were small and contained a few connective tissue elements, capillaries, and myeloid cells. The intertubular spaces between the tubules of the second type were usually much larger and contained besides the usual constituents packets of typical "luteal" cells which had a characteristic histological appearance (fig. 2). The nuclei of these cells were oval or slightly crenated and contained several deeply stained chromatin blocks; the cell boundaries were distinct. The cytoplasm was vacuolated and presented the clear appearance so characteristic of "luteal" cells in the fowl after fixation with Bouin and due to the solution of the infiltrated fat.

It should be noted that this bird was regarded by Finlay as a normal male with full cocky plumage. There was no evidence of henny feathering due to the presence of the islets of "luteal" cells in the interstices between the tubules of the testis graft.

(*b*) *Fowl No.* 10.—The left vas deferens was ruptured close to its junction with the testis when eleven days old. Killed for examination at one year old. *Histology.*—Both testes were in active spermatogenesis. The central areas of the tubules were filled with fully developed spermatozoa. No difference in histological appearance between the right and the left testis could be found.

Group 3. Males with Ovaries—*Fowl No.* 3.—Castrated when six days old and two ovaries from sisters, engrafted one on each side. Killed when eleven months old. On examination there was found on the left side a spherical testis nodule, 1.5 cm. in diameter, attached to the last intercostal muscles. Just anterior to the kidney was an ovary about one-half the usual size and apparently normal macroscopically, with yolks in all stages of development. On the right side there was a small undeveloped ovarian graft.

Histology.—The ovarian graft from the left side presented the typical structure of an ovary with an outer cortical zone containing the developing oöcytes and an inner fibrous medullary zone. It showed, however, evidence of degenerative changes in that the stroma had been infiltrated by an enormous number of granulocytes. Many of the oöcytes were normal and showed various stages in development. Follicular atresia was nevertheless extremely common. In the smaller oöcytes early stages in atresia were shown by the

thickening of the membrana granulosa which had become almost entirely detached from the thecæ owing to the shrinkage of the oöcyte. In some of the larger follicles (up to 5 mm. in diameter) the process had gone further, the cells representing remnants of the membrana granulosa having undergone fatty infiltration. A similar appearance in the cells of the inner layer of the theca interna was observed. One or two cystic follicles were found to be present.

The ovarian graft from the right side had retained the typical structure of an ovary, but degenerative changes were more advanced (fig. 3). A cluster of irregular cystic follicles of varying size projected from the surface at one end of the gonad. The thin-walled cysts contained a fluid substance in which were found a few erythrocytes and occasionally scattered lymphocytes. In addition, larger follicles in an advanced stage of atresia were found. In these the membrana granulosa had disappeared and fatty infiltration of the cells of the inner layer of the theca interna had taken place. Projecting into the cavity of the degenerating follicle at several points were strands of tissue evidently derived from the theca and containing, besides the cells impregnated with fat, numerous granulocytes. One or two small oöcytes in which no evidence of degenerative changes were observed were present. As before, the whole of the graft was invaded by enormous numbers of granular cells.

The nodule of regenerated testis was enveloped by a thick vascular connective tissue sheath similar to the tunica albuginea of the normal testis. The tubules were large and were all in active spermatogenesis. All stages in meiosis were seen and fully formed spermatozoa were found in large numbers in the centre of the tubules. As in the normal functioning testis the intertubular spaces were extremely small and contained only a few cell elements. This testis nodule showed no evidence of degenerative changes and was histologically similar in every way to the functional testis of a normal cock.

Fowl No. 15.—This bird had both testes removed two days after hatching and two ovaries from sisters implanted, one on each side. When killed at the age of fifty-two weeks it was found on examination to possess four gonad growths, as follows: (1) A large growth on the left side attached to the anterior end of the kidney; (2) A growth about the size of a pea attached to the intercostal muscles on the left side. (3) A small growth on the right side at the anterior end of the kidney. (4) A nodule of regenerated testis on the left side in the normal situation.

Histology.—Growth (1) was surrounded by an extremely thick fibrous sheath of connective tissue in the outer layers of which a few degenerate epithelial cords were found. The body of the growth consisted of a mass of proliferated undifferentiated epithelial cords separated by invading bands of fibrous tissue. The presence of many mitotic figures in the cords gave evidence of active growth. There was no indication of further differentiation of the cellular cords nor any trace of previous ovarian elements.

(2) The left intercostal growth (fig. 5) was a lobulated organ enclosed by a narrow sheath of fibrous connective tissue. The lobules were separated from one another by wide bands of invading fibrous tissue. Large clusters of cystic follicles of irregular size and varying shape were usually found at the peripheral

regions of some of the lobules. The bulk of the growth consisted of small areas of undifferentiated epithelial cords and tubules embedded in a connective tissue stroma. The tubules in the growth could be divided into two types: *Type* (*A*) – Regular tubules with a well-defined basement membrane lined by a regular layer of columnar epithelium; the nuclei of these cells resembled cytologically the nuclei of the cells of the sex cords. These tubules contained a definite lumen and were similar in appearance to the tubules found in the testes of the sexually immature cock. *Type* (*B*)—The tubules of this type were as a rule larger, more irregular in shape, and showed ramification. Lining the basement membrane there was a syncytial epithelial layer of cells with scattered nuclei which differed cytologically from those of the germinal epithelial cells lining the tubules of type (A). One or two deeply staining nucleoli and fine connecting strands of less deeply staining threads were commonly found. The cytoplasm was fibrillar and the radiating strands tended to obscure the lumen, only a small irregular cavity remaining in the centre of the tubules. The tubules of the two types occurred together both in fairly large aggregates and occasionally in smaller isolated groups scattered through the sex cord region of the lobules. Large clusters of granular cells were seen in many areas of the growth.

(3) The growth from the right side presented the same histological features as that described above. It was a lobulated organ enveloped by a sheath of fibrous connective tissue. The lobules were composed of masses of undifferentiated sex cords and tubules of types (A) and (B) embedded in a fibrous stroma. The growth was heavily infiltrated by enormous numbers of lymphocytes which were largely found in the tissue between the tubules. Many of the tubules in the central area of the lobules were filled with lymphocytes also, following the absorption of part of the basement membrane. As before clusters of cystic follicles projected from the surface of the growth.

Figs. 7 to 10 are drawn from these growths and show the various stages in the transformation of the undifferentiated sex cords into tubules of types (A) and (B). In fig. 7 a typical undifferentiated sex cord is shown. The large oval nuclei are relatively rich in chromatin and the cell boundaries are distinct. In the next stage (fig. 8) the sex cords have increased greatly in size following active mitosis. The cell outlines, easily distinguishable in the sex cord, are indefinite. Later (fig. 9) the cells form a syncytial layer lining the basement membrane, a definite lumen appears, and the tubules take on the appearance of those found in the testis of the immature cockerel. In fig. 10 a further stage is seen in which the epithelial cells lining the basement membrane present a different histological picture: the cytoplasm has become fibrillar and partially obscures the central lumen, while the nuclei are less rich in chromatin than those of type (A). While fig. 10 represents the final phase of differentiation as found in these growths, intermediate stages in the formation of tubules of type (B) from those of type (A) are frequently met with.

The testis nodule was enveloped by a thick vascular fibrous tunica albuginea. The tubules were large and packed closely together; the intertubular spaces were therefore necessarily small and the interstitial elements were similar to those found in the normal testis. There was a considerable amount of

invading fibrous tissue separating the peripheral tubules of the graft. All the tubules were atrophic (fig. 6) and histologically very similar to the tubules of type (B) described above. There was a well-developed basement membrane lined by a somewhat irregular layer of germinal epithelium with scattered nuclei containing usually a deeply staining nucleolus. The cytoplasm of the cells was fibrillar, and filamentous strands crossing the lumen almost obliterated it.

Fowl No. 16.—Both testes removed when four days old. On either side an ovary from a sister was implanted. Killed for examination when fifty-one weeks old. It was found to possess a regenerated testis nodule, about one-third the size of a normal testis. On the right and left intercostal sites were small growths.

Histology.—The intercostal growths from both sides of the bird presented the same histological structure. Each was enveloped by a layer of fibrous tissue. On the surface of the grafts clusters of cysts projected, representing the remains of degenerated ovarian follicles. The bulk of the growths was composed of masses of undifferentiated sex cords and tubules embedded in a stroma which consisted mainly of fibrous connective tissue. The central area was filled with a mass of cellular cords many of which showed evidence of degeneration. Between the sex cords could be found masses of deeply stained caseous material resulting from complete degeneration of some of the cords. Fatty infiltration of many of the sex cords had given rise to islets of typical "luteal" cells, which were more commonly found in the outer regions of the graft. Scattered tubules of both type (A) and type (B) occurred throughout the entire graft, but were more abundant in the peripheral region. Considerable infiltration of granulocytes and lymphocytes had taken place and large masses of these cells were present.

The regenerated testis nodule was similar in appearance to the normal testis in active spermatogenesis. The tubules contained cells which showed all phases of meiosis up to the formation of the fully developed spermatozoon; no evidence of degeneration was found. The intertubular spaces were rather larger than those found in the normal testis, but contained mainly spindle-shaped connective tissue elements and derivatives of the blood cells. Several of the tubules were found to contain a dense deeply stained mass of spermatozoa, and the surrounding intertubular spaces were packed with enormous numbers of lymphocytes. Such tubules were found in all cases of grafted and regenerated testis nodules in which active spermatogenesis occurs, and their presence is due to the fact that there is no connection between epididymis and spermatic tubules, so that as the sperm ripen and pass down the tubules they are retained in the occluded ends. The masses of lymphocytes were evidently actively engaged in the removal of the products of degeneration.

Fowl No. 17.—Both testes removed four days after hatching and about three-quarters of the ovary from a sister put into the left kidney site. The bird died when forty-three weeks old and was found to possess a small graft on the left side.

Histology.—The graft (fig. 11) had a typical ovarian structure with numerous well-developed follicles present; many of these were apparently normal but

both early and late stages of typical follicular atresia were found. The gonad was enclosed in a fibrous sheath. The stroma between the oöcytes was composed largely of undifferentiated sex cords and immature tubules of type (A); no tubules of type (B) were present. Ingrowth of fibrous tissue was noticeable between the aggregations of tubules. Islets of "luteal" cells were abundant both in the thecæ of the follicles and scattered through the sex cords and tubular regions. Heavy infiltration of granular cells had occurred in the peripheral layers of the graft.

In spite of the fact that this graft contained abundant "luteal" tissue, no evidence of hen-feathering had been shown during the life of the bird.

Fowl No. 18.—Both testes removed when four days old. An ovary from a sister was implanted, three-quarters on the left side and one-quarter on the right side. The bird died when thirty-one weeks old. Examination of the abdominal cavity revealed on the left side a graft about 1 cm. in diameter; on the right side there were three small gonad grafts which together were somewhat smaller than that from the left side.

Histology.—The grafts all presented the same histological appearance. There was a very thick highly vascular outer covering of fibrous connective tissue. The body of the grafts consisted of tubules of type (A) with a well-defined basement membrane lined by columnar epithelium, having a regular central lumen and embedded in a connective tissue stroma. The grafts were somewhat lobulated and the lobules were separated from one another by broad bands of fibrous tissue, invading strands of which were present between the tubules. Islets of "luteal" cells were found in large numbers between the tubules and were especially abundant in the central region. The tubules in the centre were flattened and appeared to be undergoing pressure atrophy; the nuclei of the germinal epithelium cells were more widely spaced and fewer in number than in the outer tubules; many of these nuclei were pycnotic. From the surface of the grafts clusters of irregular cystic follicles projected.

Group 3. Males with both Ovary and Testis—(a) *Fowl No.* 4.—When eleven days old one testis removed and one half of the chopped ovary from a sister inserted in the left side. The bird died from pneumonia when thirty-six weeks old. A piece of grafted ovary about 7 mm. in size was found attached to the intercostal muscle on the left side.

Histology.—The graft from this bird was fixed at some considerable period after death, and the histological preparations were therefore not very satisfactory. It was possible, however, to identify that the graft had preserved its ovarian structure but was obviously degenerate. Many ovarian follicles were present, but all the larger follicles were in an advanced stage of atresia.

Preparations of the right testis showed that the tubules were immature and resembled those of type (A), *i.e.* were small and regular, with a syncytial lining of columnar epithelium and a well-marked lumen. The testis was identical in structure with that from an immature cockerel.

(b) *Fowl No.* 20.—The details regarding the grafting operations in this bird had not been recorded, but judging by its place in the series, Finlay concludes that it was eleven days old when operated on. The bird was killed when eighteen months old, and on post-mortem examination two testis nodules were

found in the testis site on the right and left side respectively. The combined weight of the two nodules was 3·5 gms., or about one-sixth of the amount a normal cock would have possessed at this age. An ovary graft was found attached to the intercostal muscle on the right side; it was 1 cm. in diameter. At the kidney site on the left side a smaller ovarian graft, 0.5 cm. in diameter, was present.

Histology.—The right intercostal graft was a lobulated organ enclosed by a layer of columnar germinal epithelium (fig. 12). Below this there was a fibrous band of varying thickness composed of flattened connective tissue cells with spindle-shaped nuclei. The bulk of the graft consisted of masses of proliferated epithelial cords. From the periphery of the graft clusters of cystic follicles, the characteristic appearance of which has been described previously, projected. There was a well-marked medullary region with fibrous vascular cords. The structure of the graft then was that of an ovary in which consequent upon the degeneration of the follicles the ovarian stroma had become invaded by numerous cellular cords. There was some evidence that the sex cords were derived from a new proliferation from the germinal epithelium. There had been some infiltration of granular cells in the peripheral region. Scattered between the masses of undifferentiated sex cords there were small aggregates of tubules with a definite basement membrane lined by a regular layer of columnar epithelium and possessing a definite lumen (type A) (fig. 13). The tubules showed little evidence of convolution or branching. Fatty infiltration of many of the sex cords had given rise to numerous islets of typical "luteal" cells. The sex cords in the peripheral region were actively growing as was evidenced by the presence in them of numerous mitotic figures. In the central regions the sex cords did not show signs of active growth and many of them were degenerating. The final stage in the process of degeneration was represented by the occurrence between the cords of masses of deeply staining amorphous material.

The ovarian graft from the kidney site on the left side, though it presented in the main a histological appearance similar to the graft just described, was different in one or two respects. It was encapsuled in a broad band of vascular fibrous tissue. As in the former, no normal oöcytes were present, but clusters of cysts of varying size projecting from the periphery represented the degenerated remains of the ovarian follicles. Lobules containing masses of proliferated cellular cords were present, but no subsequent differentiation of the cords into tubules had occurred. Fatty infiltration of many of the cords had led to the formation of numerous islets of typical "luteal" tissue. In this growth, hypertrophy of the connective tissue elements between the cellular cords was noticeable. Many of the cords were degenerate. As in the previous graft aggregates of granular cells were prominent especially in the peripheral region of the growth.

The regenerated testis from the left side was surrounded by a fibrous tunica albuginea. Two types of tubules were present, namely (1) large swollen tubules in active spermatogenesis packed closely together with small intertubular spaces: large numbers of fully formed spermatozoa were found in the central area of the tubules, which differed in no way from those of the normal testis

actively engaged in the production of spermatozoa. (2) Tubules of the second type were smaller, inactive, and occurred chiefly in the peripheral region of the gonad. These had a characteristic appearance. Lining the basement membrane there was a layer of columnar epithelial cells the nuclei of which contained one or two deeply staining nucleoli and a scanty reticulum of very pale threads. The cytoplasm was fibrillar and in the smallest tubules completely obliterated the lumen. These tubules were identical in structure with those found in many of the ovarian grafts and designated type (B). Between these tubules the spaces were larger than usual and were occupied by comparatively large islets of typical "luteal" cells (fig. 14). In the centre of the graft there were two small groups of tubules packed with masses of fully developed spermatozoa, and still retaining the lining of germinal epithelium although there was no evidence of active spermatogenesis.

The regenerated testis from the right side presented a somewhat different histological appearance as compared with the one from the left side in that all the tubules present were of the second type. The cytoplasm of the cells of the germinal epithelium was fibrillar, the lumen when present was irregular. In many of the tubules it was either wholly or partially obliterated by the cytoplasmic strands. The intertubular spaces were large and contained besides the intertubular elements normally present packets of cells heavily infiltrated with fat and identical with the "luteal" tissue usually found in the ovary of the fowl. There was no evidence of spermatogenesis.

(c) *Fowl No.* 21.—When six days old the left testis was removed and several pieces of ovary from a sister implanted; it died when nearly ten months old. Post-mortem examination revealed on the right side a small testis weighing 180 mgms.; there was only a tiny ovarian graft weighing about 30 mgms.

Histology.—The ovarian graft presented the structure of a typical ovary. It consisted of cortex and fibrous medullary cords. Numerous normal follicles in various stages of development were present. Many of the larger follicles were in an advanced stage of atresia. Islets of "luteal" cells were conspicuous in the thecæ internæ surrounding the oöcytes. There was no infiltration of granular cells.

The right testis presented a structure typical of the immature gland. The tubules were small and lined by a single layer of columnar epithelial cells. The lumen was well defined and regular. The intertubular spaces were large and the intertubular tissue elements numerous.

Group 4. Females with Testes—(a) *Fowl No.* 5.—Ovariotomised when four days old and two testes from a brother implanted one on each side. Killed when fifty-one weeks old. Two very large testis growths were found, one on each side. No evidence of ovarian regeneration.

Histology.—The grafts were surrounded by a thick fibrous vascular tunica albuginea and there was considerable ingrowth of fibrous connective tissue between many of the peripheral tubules. The tubules were in active spermatogenesis and differed in no way from those found in the active testis of a normal cock. In several regions of the grafts small groups of very large swollen tubules containing a dense mass of ripe spermatozoa were found

(fig. 15). The basement membrane of these tubules was somewhat thickened. The surrounding intertubular spaces were larger than usual and were packed with lymphocytes. Small raised vesicles on the external surface of the grafts were also found to be filled with ripe active spermatozoa.

(b) *Fowl No.* 22.—Ovariotomised when seven days old and four testes from brothers implanted, two on each side Accidentally killed when six months old. It had a testis nodule about 8 mm. in diameter and a piece of regenerating ovary about the same size. The latter was not made available for histological examination.

Histology.—The testis nodule was enveloped by a sheath of fibrous connective tissue—the tunica albuginea. The tubules were small and of an immature type, *i.e.* lining the basement membrane was a definite layer of columnar germinal epithelium. The tubules contained a definite lumen. The intertubular spaces were large, as is normally found in the testis of an immature bird, and the elements contained therein were normal.

(c) *Fowl No.* 23.—Ovariotomised when six days old (?). Four chopped up testes from half-brothers were implanted into the kidney sites. Died when nearly nine months old. Two testis grafts were found, one at the left kidney site and the other over the left adrenal. The combined weight of the grafts was 0.7 gm. There was a persistent nodule of ovarian tissue about the size of a pin's head, which was not available for histological examination.

Histology.—The testis nodules were surrounded by a thick fibrous tunica albuginea. The tubules present were of two types: (1) large swollen tubules in active spermatogenesis, which comprised the bulk of the graft; (2) somewhat smaller tubules with a syncytial lining of germinal epithelium, the component cells of which had projecting fibrillar cytoplasmic processes that nearly obliterated the lumen. These tubules occurred in small scattered groups. The intertubular spaces between them were large and filled with lymphocytes. The spaces between the active tubules were of normal size and contained tissue similar to that in the normal active testis.

(d) *Fowl No.* 24.—Ovariotomised two days after hatching; one chopped up testis from a brother implanted into the kidney site. It was killed for examination when seventeen months old and a piece of grafted testis was found about 2 cm. in diameter. There was a regenerating ovary in its normal situation. The base of this gland had a typical ovarian appearance, but there was a projecting new growth in the posterior region almost as large as the testis graft.

Histology.—Sections of the anterior end of the ovary showed that it was surrounded by a continuous sheath of fibrous tissue. The body of the graft consisted of masses of proliferated sex cords embedded in a fibrous stroma. Many of the cords in the central area were degenerating, and the final stage in this process was marked by the presence of masses of deeply staining caseous substance between adjacent cords. The presence of mitotic figures indicated active growth in the peripheral cords. There was no evidence of further differentiation of the cellular cords into tubules. There was a large amount of "luteal" tissue present and islets of "luteal" cells formed by the fatty infiltration of the cellular cords were prominent throughout the gonad.

Embedded in the mass of sex cord tissue there were many small apparently normal oöcytes, the nuclei of which were in the diplotene stage (fig. 16). From the surface of the gonad numerous projecting cysts containing a thin fluid represented the ultimate degeneration of many of the follicles. There was a fairly heavy infiltration of granulocytes in the outer region of the gonad.

Histological examination of the new growth from the posterior region of the regenerated ovary revealed a highly fibrous structure. There was a very thick fibrous outer coat and a stroma consisting largely of fibrous connective tissue in which masses of typical sex cord tissue were embedded. As was the case in the anterior portion of the gonad, there was no evidence of tubule formation, but many of the cellular cords were greatly enlarged and showed signs of active growth. There was consideraole degeneration of many sex cords in the central region. No ovarian follicles, either normal or degenerate, were found.

The structure of the testis graft was histologically similar to that found in the bird described previously (fowl No. 23). Most of the tubules were large and all stages of spermatogenesis were present, the central areas of the tubules being filled with masses of fully developed spermatozoa. The intertubular spaces were small and contained tissue similar to that found in the testis of the normal cock. Scattered among these tubules were smaller inactive ones, identical with those which have been previously designated type (B). The interstices between these tubules were much larger and contained besides connective tissue elements large numbers of lymphocytes.

Group 5. Females without Gonads—(a) *Fowl No.* 6.—Ovariotomised when two days old. Killed for examination fifty-two weeks after the operation. Post-mortem examination revealed on the right side at the ovarian site a somewhat flattened gonad resembling in appearance the ovary of a month old chick. It was about 0.5 cm. in diameter. There was no regeneration of ovary on the left side.

Histology.—The gonad consisted of lobules filled with tubules and enveloped by a very vascular connective tissue sheath resembling the tunica albuginea of the normal testis (Fig. 17). The presence of definite female sex cells could not be demonstrated. The structure of the gonad was undoubtedly testicular; the tubules belonged to either of the two types described previously. Type (B) (fig. 21), large branching tubules with a somewhat scanty epithelium lining the basement membrane; the fibrillar protoplasm of these cells completely obliterated the lumen. This type of tubules occurred chiefly in the peripheral regions of the gonad. Tubules of type (A) (fig. 18), occurred in the central area of the gonad. They were small, packed closely together, and lined by a single layer of columnar epithelial cells. A definite central lumen was present in the majority of the tubules and mitotic figures were frequently met with. In their general appearance they resembled the tubules found in the testis of a normal immature cock (fig. 19).

Between the lobules tubules of a different type were found. They were separated from one another by fairly dense fibrous connective tissue, and were characterised by the presence of a wide irregular central lumen (sometimes filled with deeply staining debris) and a rather shallow columnar epithelial lining (fig. 20). They resemble parovarian rather than spermatic tubules. There

is, however, a possibility that they are similar in nature to the tubules found in the lobules, and the difference in structure may be attributed to the fact that they are growing in looser tissue.

That the tubules of type (B) are derived from those of type (A) is very well shown in this gonad. In many cases type (A) and type (B) were both represented in one section of a tubule, one end possessing a compact epithelial lining and a definite lumen, while at the other end the epithelial cells were scattered and the protoplasmic strands projecting from them completely obliterated the lumen. No meiotic stages were present in any of the tubules.

(b) *Fowl No.* 26.—Ovariotomised seven days after hatching. Killed for examination forty-seven weeks after the operation. Post-mortem examination revealed on the right side at the gonad site an elongated lobulated gland, about 13 mm. long × 3 mm. broad. There was no trace of any gonad tissue on the left side.

Histology.—Examination of the gonad proved it to be an ovo-testis (fig. 22). The whole organ was enveloped by a fibrous vascular sheath of connective tissue; the part covering the ovarian portion of the gonad was thin, while that covering the testicular portion was greatly thickened and resembled the tunica albuginea of the normal testis.

The ovarian portion of the gland presented the typical structure of an ovary, namely a cortical zone composed of dense stroma, and an inner fibrous medullary region. The female sex elements were represented by normal follicles in various stages of development occurring throughout the cortical zone. On comparison with the oöcytes from a functional ovary of a normal hen, no appreciable difference could be detected. The number of normal oöcytes present was not very great. Follicles in various stages of degeneration were found. The ultimate stage of follicular atresia was represented by masses of hyaline fibrous tissue and of adipose tissue. The larger degenerate follicles projected from the periphery of the gonad, while the smaller follicles were found embedded in the outer layer of the stroma. Most of these were filled with a semi-fluid substance in which a few nests of degenerating cells were found. In some of the smaller cystic follicles a knob of typical sex cord tissue had penetrated the capsule and appeared quite healthy. However, no later stages in the process of transformation of this tissue into definite testicular elements, as recorded by Fell, were encountered. "Luteal" cells were usually found in the thecæ internæ surrounding the oöcytes, and were also present in islets distributed throughout the undifferentiated sex cord region. The testicular portion of the gonad was represented by large lobules composed of tubules which could again be divided into the two types (A) and (B) as previously described. Tubules of a type intermediate between (A) and (B) were also found. The intertubular tissue consisted mainly of small aggregations of connective tissue elements, although large and small islets of typical "luteal" cells were present. In addition to the elements already described, large areas of undifferentiated sex cords in active mitosis were present. In none of the tubules of testicular appearance was there any indication of the onset of spermatogenesis.

Group No 6. Females with both Ovary and Testes—(a) *Fowl No.* 8.—Two chopped-up testes from a brother were implanted seven days after hatching. Part of the graft was placed subcutaneously under the wing. Killed when forty-seven weeks old. On post-mortem examination, two testis grafts were found in the body cavity, one attached to the membrane of the air sac and the other attached to the intercostal muscles. Two subcutaneous grafts, elongated in shape and measuring 1.5 cm. × 0.5 cm. were also found. The ovary from this bird was not made available for histological examination, but macroscopically appeared to be normal. The bird had laid normally; one of her eggs had been incubated and proved fertile.

Histology.—The subcutaneous portions of testis graft and the intercostal graft presented the same histological features and will, therefore, be described together. The grafts were enveloped by a characteristic tunica albuginea. The tubules were large and packed closely together, the intertubular spaces were therefore necessarily small and contained the usual constituent elements as found in a normal testis. Nearly all the tubules were in active spermatogenesis and ripe spermatozoa were plentiful in the central area. A few of the tubules at the periphery of the grafts were in a state of reduced activity; comparatively few meiotic stages were present, but the basement membrane was lined by a deep layer of spermatogonia. Swollen tubules packed with enormous numbers of ripe spermatozoa were present. The intertubular spaces surrounding these tubules were comparatively large and contained masses of lymphocytes. Lymphocytes were also frequently met with in the space adjacent to the tubules in which degeneration was apparent.

The graft attached to the membrane of the air sac (fig. 23) differed from the normal testis in active spermatogenesis only in that the intertubular spaces were much larger. The interstitial elements were normal.

(b) *Fowl No.* 29.—One half of the ovary was removed and two testes, one from a brother the other from a half-brother, implanted at the left kidney site, and one testis from a half-brother implanted at the right site when eleven days old. Post-mortem examination revealed on the left side a single piece of grafted testis, about 2 cm. in diameter. The ovary was not made available for examination, but it had functioned normally during life and eggs had been laid; when the bird was killed a yolk was found entering the oviduct.

Histology.—The testis graft was composed mainly of large apparently normal tubules in active spermatogenesis. Scattered through the large active tubules were small inactive ones which contained in the central lumen masses of degenerating cells of the meiotic stages. The tubules were lined by a deep layer of spermatogonia. No atrophic tubules of type (B) were present. The intertubular spaces were small as in the normal active testis. There was a considerable ingrowth of fibrous tissue between the tubules at the periphery of the graft.

(c) *Fowl No.* 30.—Three-quarters of the ovary removed when four days old: two testes from a brother implanted, one in each side. When killed, it was found to possess on each side a large testis graft, almost the size of a functional testis of a cock. The ovary was small and no large yolks were present.

Histology.—Microscopical examination of the ovary showed that the typical

ovarian structure had been retained. There was an outer cortical zone in which the oöcytes were found embedded in the stroma, and an inner fibrous medullary zone. The oöcytes were numerous and of varying sizes. Many of the larger follicles were undergoing atresia, the cavity being filled with numerous scattered cells with highly vacuolated cytoplasm, which were apparently derived from the membrana granulosa. Islets of "luteal" cells were found in the thecæ of the more developed follicles. Embedded in the stroma there were large masses of typical sex cords (fig. 24). Further differentiation of the cellular cords into tubules had not taken place. It was not possible to determine the exact origin of the cellular cords. In some regions it appeared that they were derived by proliferation from the germinal epithelium surrounding the gonad; while in others it seemed as though there was a possibility of their being formed from the proximal portions of the canaliculised medullary cords, because numerous small epithelial cords were found in this region. Groups of granular cells occurred throughout the peripheral region of the cortex and were also very numerous in the medullary zone.

The testis graft was enveloped by a typical tunica albuginea. For the most part the tubules were large and in active spermatogenesis, numerous ripe spermatozoa being present in the central areas of the tubules. Tubules of type (B), smaller in size, occurred mainly in the peripheral region of the graft. The lumen in most of these tubules was almost obliterated by fibrillar protoplasmic strands from the epithelial cells lining the basement membrane. However, in some cases a distinct lumen was present and usually contained a few spermatozoa. Large swollen tubules packed with masses of ripe spermatozoa were found in the centre of the graft. As was previously noted in many of the other testis grafts the intertubular spaces were larger than usual and contained numerous lymphocytes.

4. Summary of Histological Results.

1. **Structure of the Grafted and Regenerated Ovarian Tissues.**—From the description of cases it is seen that, according to its histological structure, the ovarian tissue implanted into a male or the regenerated ovarian tissue in an incompletely ovariotomised female, can be conveniently grouped into the following three classes :—

(*a*) That in which the typical ovarian structure has been retained, although considerable degeneration is evidenced by the presence of many follicles undergoing atresia; many normal oöcytes are present. Nos. 3, 4, 21.

(*b*) That in which, in addition to the normal female sex elements, new proliferated sex cords are found, these in certain cases having continued their differentiation into tubular formation. Nos. 17, 20, 24, 30.

(*c*) That in which the female sex elements are represented solely by degenerated remains of the follicles, taking the form of thin-walled cysts projecting from the surface of the gonad; no normal oöcytes are present. Sex cords and the further differentiation of these into tubules are found in the stroma. Nos. 15, 16, 18.

2. **Structure of the Grafted and Regenerated Testicular Tissues.**—Most of the testicular tissues examined are in active spermatogenesis. Three of the fowls, Nos. 4, 21, and 22, were killed or died at a comparatively early age, and histological examination shows that the testes are still of the type characteristic of the immature cockerel. The tubules are small and lined by a regular layer of columnar germinal epithelium. There is no evidence of the onset of spermatogenesis.

The regenerated testis nodule from the right side of fowl No. 15 is composed of atrophic tubules of type (B), that is, tubules with a somewhat scattered lining of germinal epithelium. Fibrillar strands of cytoplasm from the epithelial cells almost obliterate the lumen. There is no evidence of spermatogenesis. The right testis from fowl No. 20 is also composed wholly of atrophic tubules of the same type.

All the testes, grafted or regenerated, from the other birds in the series, show either all the tubules present in active spermatogenesis, or a mixture of active and atrophic tubules.

3. **Structure of the Activated Right Gonads in Ovariotomised Hens.**—Of the two activated right gonads described, that from fowl No. 26 possesses the structure of an ovary in which a secondary invasion of sex cords had occurred. Tubules derived from a further differentiation of the invading sex cords are present as well as the normal female sex elements. The right gonad from fowl No. 6 is typically testicular in structure.

5. Discussion.

a. **Formation of Atrophic Tubules in the Testes.**—Apart from environmental conditions which may, in some few cases, be held responsible for the production of atrophic tubules in the testis, there is, as evidenced by the majority of the cases, a mechanical factor involved. It would appear that the accumulation of ripe spermatozoa in the occluded ends of the tubules leads to an

intratubular pressure resulting in a gradual cessation of spermatogenic activity within the tubule. This interpretation is supported by the fact that in many of the testes there are found together tubules in active spermatogenesis; inactive tubules, type (B), with numerous spermatozoa in the lumina, a few early meiotic prophases in cells lining the basement membrane, and nearer the centre degenerating cells in later stages of meiosis; and large tubules filled with masses of ripe spermatozoa. Nonidez found that retention of the sperm in the testis of the fowl following vasoligation resulted in a local arrest of spermatogenesis, which was probably due to the increased intratubular pressure and the production of noxious substances during degeneration of the sperm and immature sex cells within the seminiferous tubules. Moreover, atrophy may be caused by external pressure exerted by many enlarged active tubules which prevents the smaller tubules from functioning normally; or by a thickening of the tunica albuginea which does not permit of growth and differentiation proceeding beyond a certain stage.

b. **Luteal Cells in Testis and Ovary.**—Islets of typical "luteal" cells are found in the testis of fowl No. 9 (subcutaneous testis grafts) and fowl No. 20 (regenerated testis). These cells occur in the intertubular spaces between the inactive tubules. It is interesting to note that in fowl No. 9, a castrated male with testis grafted, the presence of "luteal" cells in the graft was not associated with a plumage of the henny type: up to the time of killing the plumage had remained typically cocky. It will be remembered that Morgan had suggested it is the secretion from these cells present in the testes of the Sebright and of the henny-feathered Campine cock which inhibits the development of cocky plumage. However, Pease from an examination of a number of birds formed the conclusion that the amount of "luteal" tissue in the testis depends on the stage of spermatogenesis, and is not associated with the type of plumage of the bird. He found that when spermatogenesis is active no "luteal" cells are found either in henny-feathered or in control cocks; but when material is taken from immature birds or birds whose testes were inactive, or spermatogenesis only beginning, "luteal" cells are always present in birds of

both series. The findings of Pease were confirmed later by Nonidez, and the same conclusion has been reached in the present investigation. No "luteal" cells are present between the tubules in active spermatogenesis, but between the inactive tubules in the testes of fowl No. 9 and fowl No. 20, islets of typical "luteal" cells are abundant.

In the normal ovary, islets of "luteal" cells occur in the thecæ of the follicles, and the same is found in the ovaries examined in this series wherever normal oöcytes are present. "Luteal" cells are also found in many of the ovarian grafts in the regions of the undifferentiated sex cord tissue and between the tubules. They are formed by fatty infiltration of many of the young sex cords. It should be noted that in fowl No. 17 a castrated male with an ovarian graft, the presence of abundant "luteal" cells in the graft did not give rise to any indication of hen-feathering during the life of the bird, the plumage remaining typically cocky.

c. **The Origin of the Sex Cords.**—It has not been possible to determine with exactitude the origin of the sex cords in the grafts. In fowl No. 30 (typical ovary with invasion of sex cords into the ovarian stroma) proliferation of new cellular cords was not very active at the time of death. The presence of one or two small invaginations from the germinal epithelium appeared to indicate a possible place of origin of the new sex cords. In the same gonad at the distal portion of the medullary cord region, aggregations of small cellular cords appear to suggest a possibility of their being formed from the distal ends of the degenerating medullary cords, although the actual process of transformation cannot be followed. Fell in her histological studies of the gonads of intersexual birds, which were in many respects similar in structure to the ovarian grafts described in this paper, was able to show definitely that the sex cords are sometimes proliferated from the peritoneal epithelium covering the gonad. She admits the possibility that the remnants of the medullary cords may take part in the formation of the epithelial cords, since conditions which stimulate the germinal peritoneum might be assumed to favour also the further development of its intra-ovarian derivatives.

There can be no doubt that the tubules found in the ovaries

of the birds described are derived from the new proliferation of the sex cords, as is shown by figs. 7 to 10. In none of the cases described does the sex cord tissue give rise to mature seminiferous tubules and there is no evidence of spermatogenesis. The tubules of type (A) are similar to those found in the immature testis of the normal cock, while tubules of type (B) are similar to those atrophic or inactive ones found in the grafted and in the regenerated testis. In some of the gonads of the intersexual birds described by Fell, tubules of the same two types were present. That the tubules of the embryonic type were spermatic and could under favourable conditions give rise to the definite sex elements characteristic of the male was shown by some of the intersexual gonads, where further differentiation of the immature tubules had taken place, resulting in active spermatogenesis.

It is probable that the birds in this series had not been kept long enough for the tubules to become mature, but from the evidence given above it can be concluded that they are spermatic and would, under favourable conditions, further differentiate into mature tubules in active spermatogenesis.

d. **Factors effecting Structural Changes in the Grafted Ovaries.**—The results of histological examination have shown that many of the implanted ovaries exhibit new growth in the form of proliferated sex cords which, in their further differentiation, develop into definite spermatic tubules. Similar structures were found in the gonads of a number of intersexual birds described by Crew and by Fell. The ovaries from these birds had functioned normally but a change in the physiological environment, ascribed by Crew to excessive egg laying, hæmorrhage, or ovarian disease, had provided a stimulus for the production of a new proliferation of sex cords.

In the development of the chick up to the seventh day there occurs a single proliferation of sex cords from the germinal epithelium; at this stage the gonads in the two sexes are identical in structure. Further differentiation gives rise to the structure typical of the female or the male gonad. In the male the sex cords of the first proliferation develop into mature spermatic tubules; in the female they degenerate, while about the eleventh day a second proliferation of sex cords from the

germinal epithelium gives origin to the oöcytes. The remnants of the degenerated cords of the first proliferation persist in the ovary, and epithelial elements derived from the medullary cords are found scattered throughout the cortex and in the thecæ of small oöcytes. The epithelial cells in the thecæ of the follicles give rise by fatty infiltration to islets of "luteal" cells.

It appears that at an early stage in the development of the embryonic ovary there is present a specific physiological environment which provides the necessary stimulus for a secondary production of sex cords from the germinal epithelium, and these, under the specific stimulus, give rise to the definite ovarian elements. These conditions obtain only at an early stage of incubation, and are apparently not normally reproduced at any phase of development subsequent to this. If, therefore, at any further developmental stage a stimulus is provided which encourages a new proliferation of sex cords, either from the germinal epithelium or possibly from the epithelial elements in the medullary region, then their further differentiation is in the direction of definite spermatic tubules. In the male no such specific physiological environment obtains, and consequently all new proliferations of the sex cords give rise to spermatic elements. The results of this investigation as well as those obtained from intersexual birds lend some support to this hypothesis, as new growth both in grafted ovaries in these experiments and in the ovaries of intersexual birds, results in the formation of spermatic tubules. It was shown in a previous paper (Greenwood, 1924) that the process of embryonic sexual differentiation is not affected by the presence of active gonad tissue of the alternative type, and in these experiments it cannot be said that the presence of testis affects the differentiation of the new sex cords in the grafted ovary.

There is considerable evidence to show that there are many factors which may stimulate a post-embryonic formation of new sex cords. In the hen, removal of the left ovary may stimulate the development of a right gonad which normally remains undeveloped. Ovarian disease, excessive egg laying or hæmorrhage causing a change in the physiological environment has also been shown by Crew and Fell to result in the production of new sex cord tissue. In the cases listed, although

comparatively few, it would seem that there exists some relation between the age of the chick at grafting and the secondary production of germinal tissue. Ovaries grafted between the second and the fourth day after hatching all showed the presence of sex cords and spermatic tubules; those grafted later than the fourth day showed, with one exception, a typical ovarian structure, there being no secondary formation of sex cords. It is not possible to determine whether this is due to merely mechanical interference, *e.g.* the younger and smaller the ovary the more difficulty is experienced in removing it without much damage, or whether it is due to the fact that the more immature the ovary the more responsive it is to changes in the internal environment.

e. **The Significance of the Right Gonad in the Fowl.**—The right gonad in the hen is normally a rudimentary organ. It has been shown, however, by various authors (Benoit, Domm, Goodale, Zavadovsky) that the effect of the removal of the left ovary in many cases is to stimulate the development of this rudimentary gland. Histological examination of such developed gonads revealed a structure typical of the testis; all stages in spermatogenesis may be present up to and including the formation of mature spermatozoa. The presence of female sex elements in such activated right gonads has not been previously described. In the case of hermaphrodite birds, many of which have been described in recent scientific literature, the right gonad when present has always exhibited the structure of a testis.

In the development of the right gonad of the female embryo there is only one proliferation of sex cords from the germinal epithelium, which retrogress at the same time as the cords of the first proliferation in the left ovary but are not replaced by a secondary proliferation. In this respect the right gonad simulates the development of the embryonic testis. Consideration of these facts has led Benoit to postulate that: "la gonade droite de la poule ne doit pas être considérée comme un 'ovaire rudimentaire droit,' ainsi qu'on l'a fait jusqu' à présent, mais comme un 'testicule rudimentaire droit.' . . . Ces faits me conduisent à une autre notion, celle de l'existence dans un même soma, de 2 germens distincts, le germen mâle et le

germen femelle, aussi rigoureseument fixes l'un que l'autre au point de vue de leur determinisme cyto-sexuel."

The fact that in fowl No. 26 typical ovarian structure is found with a secondary invasion of sex cords and their differentiated products, tubules, must lead to a reconsideration of this view. Evidence from birds of other species, *e.g.* pigeons, hawks, owls, in which there is sometimes present a right gonad typically ovarian in structure with normal follicles, appears to suggest that this gland is in reality ovarian in nature. In the case of the two birds in which activation of the right gonad occurred, we have an instance parallel to the change in structure found in many of the ovarian grafts. The new proliferation of the sex cords, whether derived from the peritoneum or from the epithelial elements of the medullary region are moulded in their further development by the internal environment. In fowl No. 6 a definite testicular structure is formed, but in fowl No. 26 the specific internal physiological environment encouraging the formation of the definite female sex elements had been exceptionally reproduced. That the conditions were transient is shown by the fact that further development of the new sex cords led to the production of definite spermatic elements.

If we accept this tentative hypothesis, then this case would lend support to the view that the right gonad in the hen, as well as the left, possesses the chromosome complex typical of the female, and that, if differentiation proceeded under the specific stimulus normally characteristic of the female chick about the eleventh day of incubation, an ovary would be formed. The fact that such environment is reproduced only very exceptionally would account for the occurrence of testicular structures in the activated right gonad.

A further series of experiments, including the critical one of implanting ovary into a female is in progress which, it is hoped, will provide sufficient evidence to verify the hypotheses put forward in this paper.

6. Summary.

1. A histological examination has been made of material from 19 cases of gonad grafts in fowls, from the series of transplantation experiments performed by G. F. Finlay.

2. While some of the ovary grafts remain typically ovarian in structure, many show extensive changes due to a secondary proliferation of the sex cords and their further differentiation into tubules of a definitely spermatic type. Most of the testis grafts are either in active spermatogenesis or are composed of an intimate mixture of active and atrophic tubules; in 2 cases they consist of atrophic tubules only.

3. It is suggested that the formation of atrophic tubules may be due, apart from environmental conditions, to mechanical pressure.

4. Islets of typical "luteal" cells are present in the interstices between the inactive tubules in the testis grafts, and are not associated with a henny type of plumage. In ovarian grafts "luteal" tissue is found in the thecæ of the follicles, as in the normal ovary, and also in the region of undifferentiated sex cord tissue and tubules.

5. It has not been possible exactly to determine the origin of the new proliferation of the sex cords in the ovarian grafts. It would appear that they may be derived from the germinal epithelium and also from the intra-ovarian epithelial elements represented by the degenerating medullary cords.

6. There appears to be some time relation involved in the formation of the secondary proliferation of the sex cords in the ovarian grafts, since (with one exception) none are found in grafts made later than the fourth day after hatching.

7. Two cases of an activated right gonad in the ovariotomised female are described, of which one shows typical ovarian structure. The hypothesis is put forward that the right gonad is female fundamentally in constitution, and if it differentiated under a specific stimulus (normally characteristic of the female chick at an early stage of incubation but exceptionally reproduced at a later stage in development) an ovary would be formed.

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8. Description of Plates.

INDEX TO LETTERING OF FIGURES.

A.F. . Atretic Follicle	G.C. . Granular Cells
B.M. . Basement Membrane	I.T. . Immature Tubules (type A)
C.F. . Cystic Follicle	L.C. . "Luteal" Cells
D.T. . Atrophic Tubules (type B)	

FIG. 1.—Fowl No. 9. Subcutaneous testis graft showing large swollen tubules in active spermatogenesis, together with smaller inactive tubules. × 100.

FIG. 2.—The same, showing islets of typical "luteal" cells present in the interstices between the inactive tubules. × 400.

FIG. 3.—Fowl No. 3. Small ovarian graft from the right side with clusters of cystic follicles and large follicles in atresia. × 15.

FIG. 4.—Fowl No. 15. Ovarian graft showing lobulated structure. Numerous spermatic tubules are embedded in the stroma. × 15.

FIG. 5.—Fowl No. 15. Section of one of the lobules at a higher magnification to show the tubules of the two types, A and B. × 170.

FIG. 6.—Fowl No. 15. Section of the regenerated testis composed entirely of atrophic tubules. × 125.

FIG. 7.—Fowl No. 15. Typical young sex cord from the ovary graft.

FIG. 8.—Fowl No. 15. Enlarged sex cord from the same section.

FIG. 9.—Fowl No. 15. A further stage of differentiation of the sex cord in the ovary graft, showing the formation of a tubule of type A.

FIG. 10.—Fowl No. 15. Final stage, resulting in the formation of tubules of type B.

FIG. 11.—Fowl No. 17, ovarian graft. The typical ovarian structure has been retained, but the stroma is permeated by proliferated sex cords and tubules, × 20.

FIG. 12.—Fowl No. 20. Ovarian graft. Clusters of cystic follicles are seen at the surface. There is a dense stroma permeated with sex cords and small groups of tubules. × 20.

FIG. 13.—A higher power view of fig. 12 to show an enlarged sex cord and tubules of type A surrounded by smaller undifferentiated cords. × 320.

FIG. 14.—Fowl No. 20. Section of the left regenerated testis showing islets of "luteal" cells. × 250.

FIG. 15.—Fowl No. 5. Section of testis graft showing large swollen tubules filled with a dense deeply staining mass of mature spermatozoa. × 15.

FIG. 16.—Fowl No. 24. Ovarian graft showing numerous small normal oöcytes embedded in a dense stroma. × 15.

FIG. 17.—Fowl No. 6. Section through the right gonad. × 15.

FIG. 18.—The same from the region at A showing tubules of type A. × 120.

FIG. 19.—Section of a normal testis from three-months old cockerel, to show similarity in the structure of the tubules to those of type A. × 130.

FIG. 20.—Fowl No. 6. Section of right gonad from region at C, showing "parovarian" tubules. × 130.

FIG. 21.—The same, section from region at B, showing the large irregular tubules of type B. × 220.

FIG. 22.—Fowl No. 26. Section of right gonad. × 15.

FIG. 23.—Fowl No. 8. Section of the testis graft attached to the air sac membrane. Although the tubules are all in active spermatogenesis, the intertubular spaces are much larger than in the normal active testis. × 130.

FIG. 24.—Fowl No. 30. Section of regenerated ovary showing masses of proliferated sex cords in the stroma. × 120.

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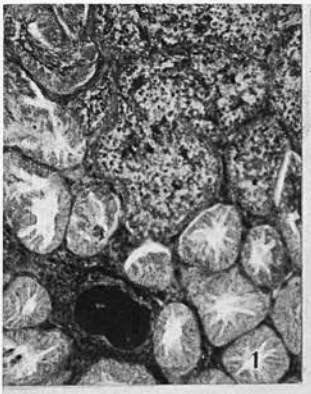


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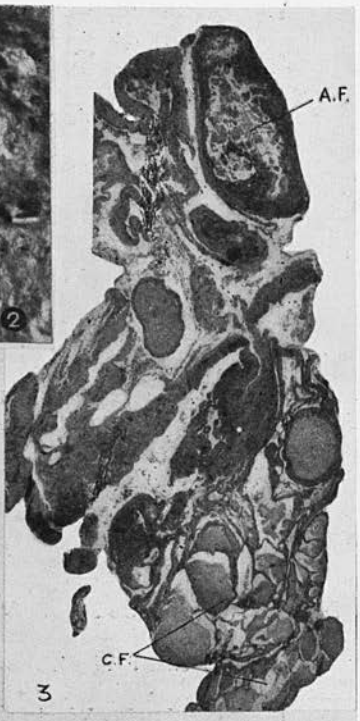
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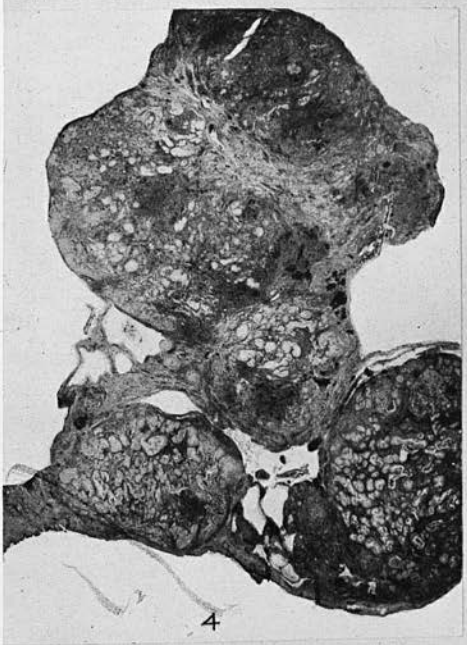

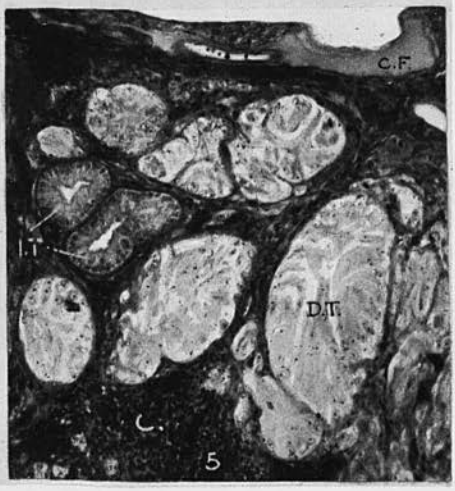
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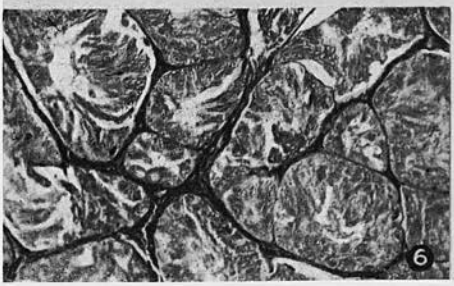

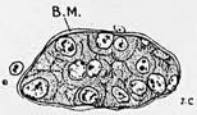

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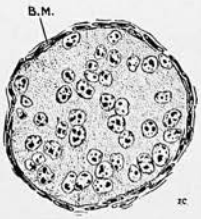

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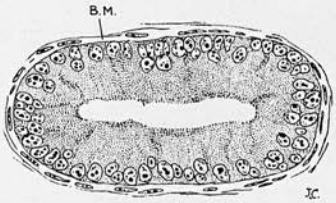

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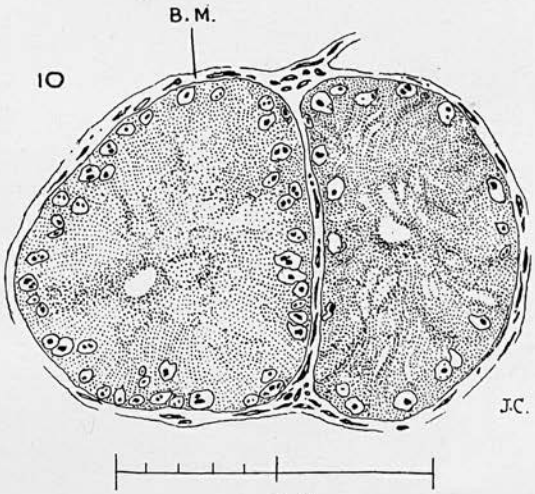

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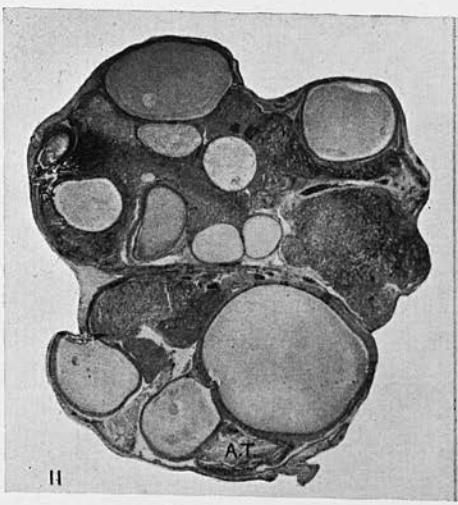

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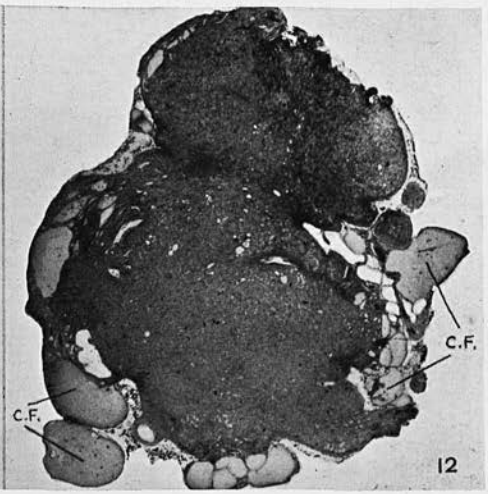

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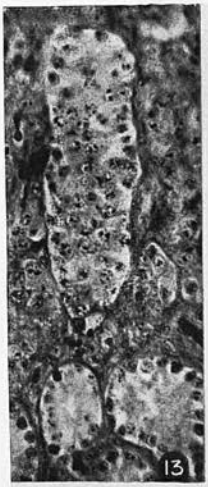

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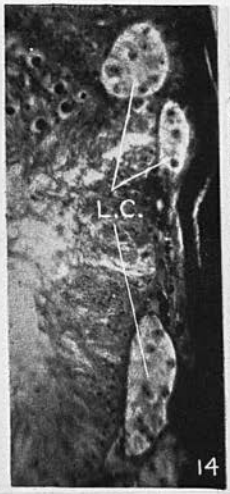

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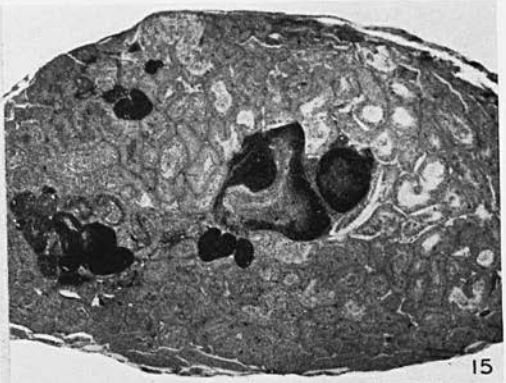

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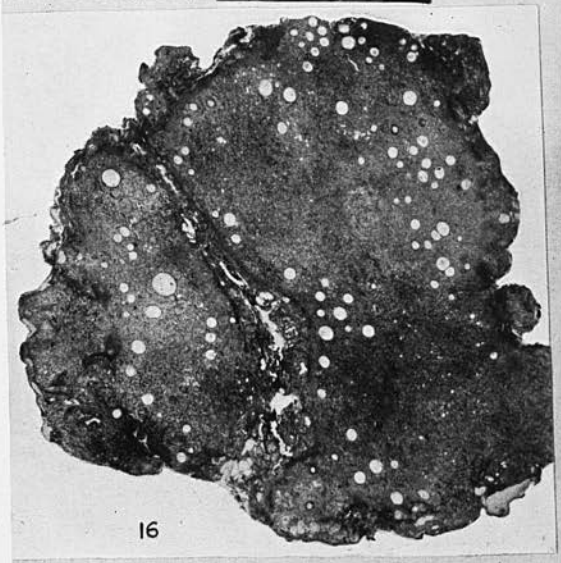

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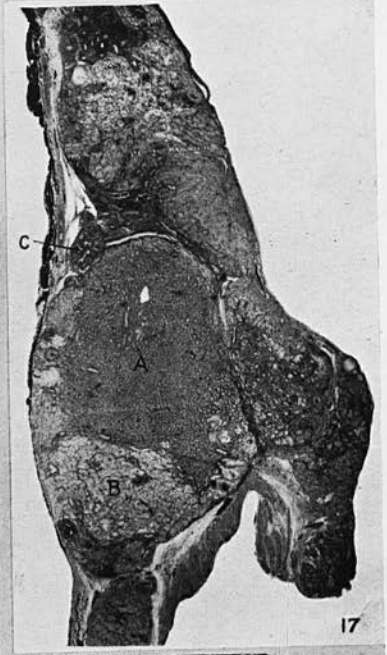

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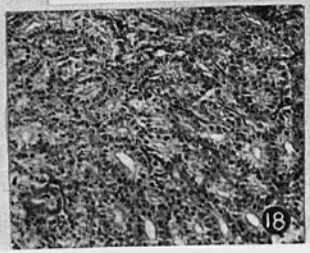

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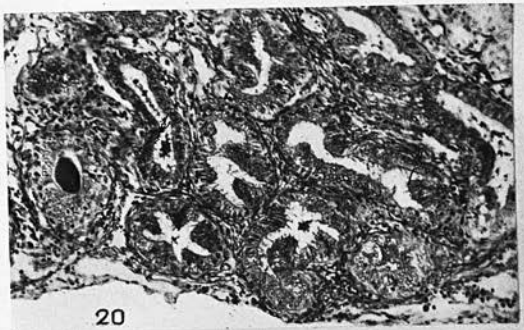

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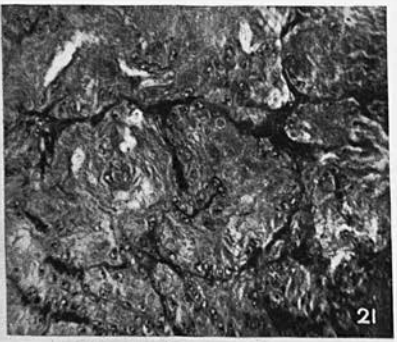

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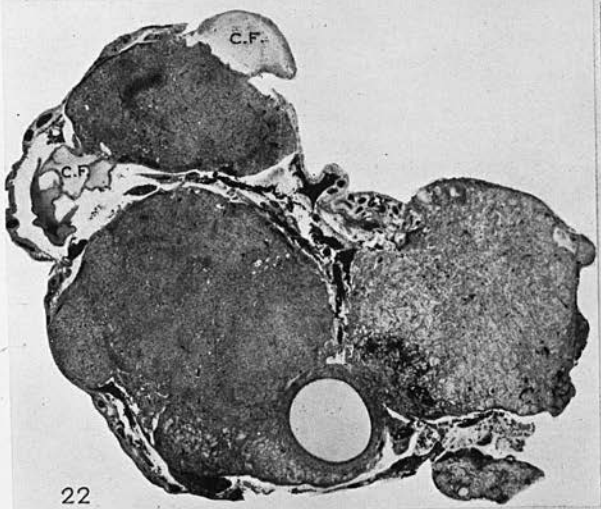

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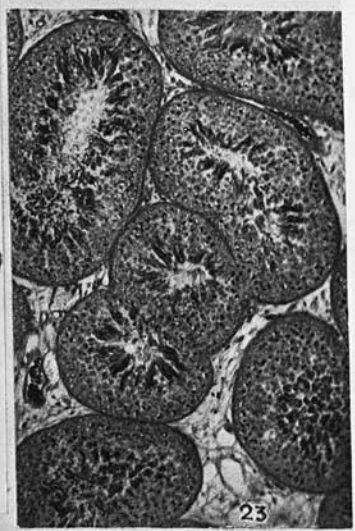

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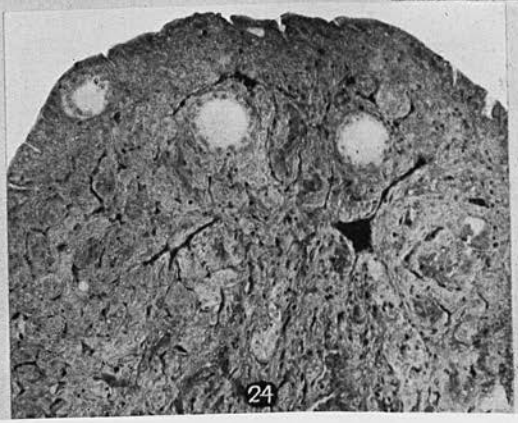

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THE GROWTH RATE IN HYPOPHYSECTOMISED SALAMANDER LARVÆ.*

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THE researches of Cushing † and others indicate that the partial removal of the pituitary gland in the young mammal inhibits normal growth. In Amphibia, Smith (1920) and Allen (1917) have shown that the early ablation of the hypophysis anlage in frog embryos produces, in addition to the characteristic pigmentary disturbances consequent on pituitary removal: (1) failure to undergo metamorphosis; (2) retardation in the development of the thyroid gland; (3) a diminished growth rate of the body as a whole.

It is not clear how far the third effect is independent of the undevelopment of the thyroid gland.

The object of the present communication is to record data concerning the growth in body weight of Salamander larvæ after the removal of the pituitary body subsequent to the development of the thyroid gland.

The material used for this experiment was the Axolotl larva of *Amblystoma tigrinum*, the European strain of which does not undergo metamorphosis if kept in aquaria.

The operations were performed by Dr Lancelot Hogben by the technique developed by him, and described in a previous issue of this *Journal*.

In all, thirteen individuals were operated on, their ages ranging from six months to one year. A corresponding series of controls of similar age was used. The animals were kept singly in jars of water, and were weighed fortnightly for a period of eighteen weeks subsequent to the operation. For the first seven weeks the animals (operated and controls) were

* Received March 4th, 1924.

† Dott, N. M. (1923), "An Investigation into the Functions of the Pituitary and Thyroid Glands," *Quart. Journ. Exp. Physiol.*, **13**, Nos. 3, 4.

TABLE I.

	Oct. 1.	Oct. 15.	Oct. 29.	Nov. 12.	Nov. 26.	Dec. 10.	Dec. 24.	Jan. 7.	Jan. 21.	Feb. 5.
					Control Series.					
C_1	14·5	15·0	15·75	16·75	16·0	15·0	17·0	17·5	17·25	16·75
C_2	6·25	8·0	7·5	8·0	9·0	9·0	9·5	10·25	9·0	9·5
C_3	15·5	17·0	15·0	16·25	17·5	18·5	20·0	20·0	22·25	23·75
C_4	19·5	21·5	21·0	21·25	22·5	23·0	24·5	24·0	24·25	24·0
C_5	5·5	6·5	6·0	6·0	6·75	7·0	7·5	7·5	8·0	8·0
C_6	6·5	7·0	6·5	7·0	8·25	8·25	8·75	8·75	9·25	9·25
C_7	5·0	6·0	5·5	5·5	6·25	6·5	7·0	7·5	7·5	7·5
C_8	6·5	7·5	7·5	7·5	8·5	8·5	8·5	8·0	8·5	8·5
C_9	5·5	6·5	6·0	6·25	7·25	7·5	7·5	8·0	8·25	8·25
C_{10}	5·0	5·75	5·75	6·0	7·0	7·0	7·5	7·5	8·0	8·25
C_{11}	6·0	7·5	7·25	7·5	8·75	8·75	8·75	9·0	9·25	9·75
C_{12}	4·75	5·25	5·25	5·75	6·5	6·5	6·0	5·75	6·0	6·0
C_{13}	5·5	6·5	6·75	7·0	8·5	8·25	8·5	8·5	9·0	8·5
					Hypophysectomised Series.					
H_1	15·5	17·5	17·0	16·5	18·0	18·5	19·0	18·5	19·75	19·5
H_2	6·5	7·5	7·0	7·5	8·0	8·5	8·5	8·5	8·75	9·0
H_3	7·0	9·0	9·0	9·0	10·0	10·5	10·0	9·5	10·5	10·5
H_4	13·25	13·5	12·5	12·75	12·5	dead	...	...	...	...
H_5	7·5	9·0	8·5	8·75	9·75	9·75	9·25	8·75	9·25	9·75
H_6	5·0	5·75	5·5	6·25	6·5	6·75	7·25	7·0	6·75	7·0
H_7	5·5	6·0	6·0	6·5	7·0	7·0	7·25	7·0	6·75	7·0
H_8	5·75	7·5	7·0	7·25	8·0	8·0	8·0	dead	...	...
H_9	6·0	6·5	6·5	7·0	7·5	7·75	8.25	8·0	8·0	8·0
H_{10}	7·0	7·5	7·0	7·0	8·5	9·0	8·0	8·5	8·75	8·5
H_{11}	6·25	8·0	7·75	8·0	9·0	9·0	9·5	9·25	9·5	9·5
H_{12}	9·5	11·5	11·25	11·5	12·25	13·0	12·5	12·5	13·0	12·75
H_{13}	4·0	5·0	5·0	5·5	5·75	6·0	5·5	5·5	5·75	5·75

TABLE II.

$\frac{\text{Final}}{\text{Initial}}$ weight ratio :—

	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}
Series C =	1·16	1·52	1·53	1·23	1·60	1·42	1·5	1·31	1·50	1·65	1·62	1·26	1·55

Mean value 1·45 σ_M = 0·049.
Coefficient of variation = 10·4.

$\frac{\text{Final}}{\text{Initial}}$ weight ratio :—

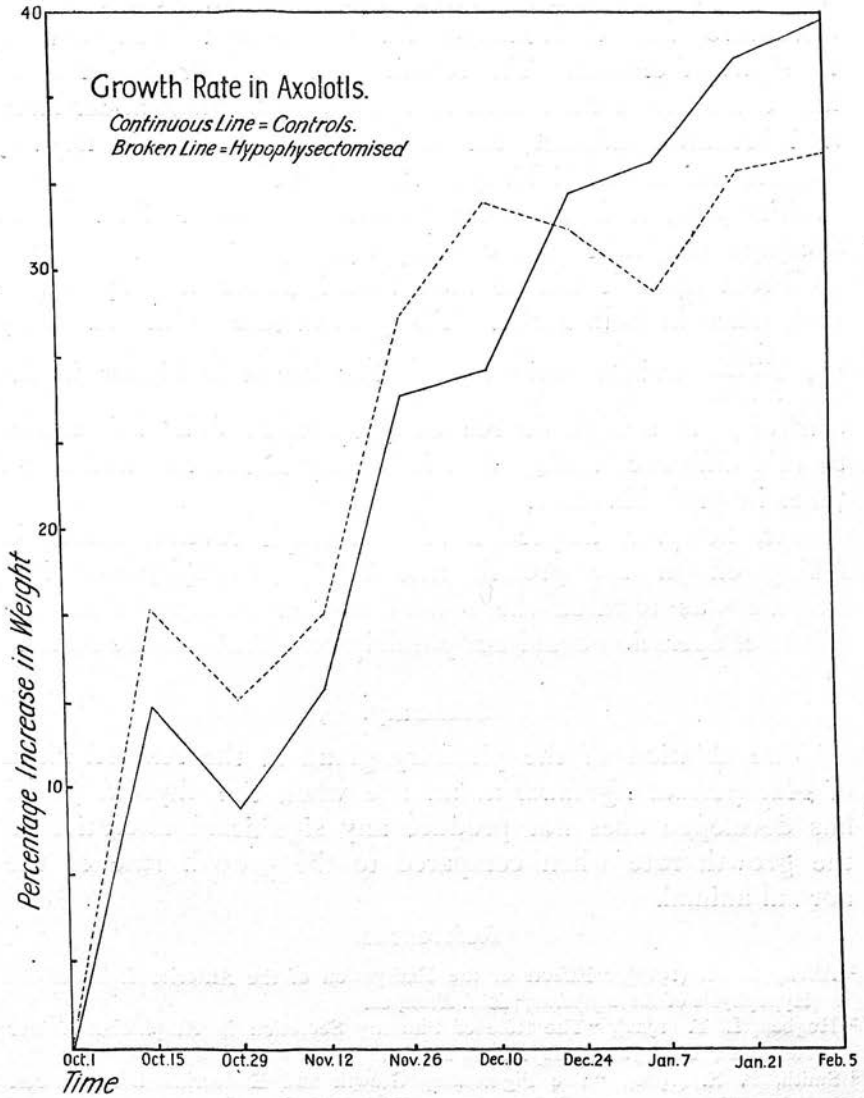
	H_1	H_2	H_3	H_4	H_5	H_6	H_7	H_8	H_9	H_{10}	H_{11}	H_{12}	H_{13}
Series H =	1·26	1·38	1·5	...	1·30	1·40	1·27	...	1·33	1·21	1·52	1·34	1·44

Mean value 1·36 σ_M = 0·029.
Coefficient of variation = 6.

Difference between Mean $\frac{\text{Final}}{\text{Initial}}$ weight ratio of two series = 0·09.

Standard deviation of the difference = $\sqrt{(0·029)^2+(0·049)^2}$.
Significance = about 1·8σ.
= about 2·7 times the probable error.

fed three times a week on raw beef, and subsequently the animals were fed daily on fresh ox liver for the duration of



the experiment. This change of diet was suggested by experimental feeding of Salamanders by Uhlenhuth, who found that ox liver favourably influenced the rate of growth.

The experimental series displayed no evident disturbance as the result of the removal of the gland apart from the

characteristic pallor described by Hogben. This was maintained throughout the course of the experiment. Two animals of the operated series died, in one case through misadventure owing to breakage of the container. They showed no signs of œdema. The remainder took their food readily, and there seems no reason to doubt that in Salamander larvæ as in Anuran tadpoles, the removal of the gland in no way impairs the normal viability of the animal.

The observations on the growth rate are set forth in the accompanying tables and chart (pp. 76, 77).

From these it will be seen that increase in body weight took place in both series. The growth rate as measured by the $\frac{\text{final}}{\text{initial}}$ weight ratio in the two series is higher in the controls, but a high degree of significance does not attach to this difference seeing that it is only about two and a half times its probable error.

As, however, inspection of the graph reveals, the main falling off in the growth rate of the hypophysectomised animals was towards the end of the experiment, when the effects of operation could not possibly contribute to the result.

Summary.

The ablation of the pituitary gland in the Axolotl larva of *Amblystoma tigrinum* at an age when the thyroid gland has developed does not produce any significant alteration in the growth rate when compared to the growth rate of the normal animal.

References.

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